Ayurvedic Pharmacopoeia of India
Part-I e-Book
Appendix Volume - I

APPENDIX-I

1. APPARATUS FOR TESTS AND ASSAYS

1.1 Nessler Cylinders

Nessler cylinders which are used for comparative tests are matched tubes of clear colourless glass with a uniform internal diameter and flat, transparent base. They comply with Indian Standard 4161-1967. They are of transparent glass with a nominal capacity of 50 ml. The overall height is about 150 mm, the external height to the 50 ml mark 110 to 124 mm, the thickness of the wall 1.0 to 1.5 mm and the thickness of the base 1.5 to 3.0 mm. The external height to the 50 ml mark of the cylinder used for a test must not vary by more than 1 mm.

1.2 Sieves

Sieves for pharmacopoeial testing are constructed from wire cloth with square meshes, woven from wire of brass, bronze, stainless steel or any other suitable material. The wires should be of uniform circular cross-section and should not be coated or plated. There must be no reaction between the material of the sieve and the substance being sifted.

Sieves conform to the following specifications –

Approximate sieve number*	Nominal mesh aperture size mm	Tolerance average aperture size ± mm
4	4.0	0.13
6	2.8	0.09
8	2.0	0.07
10	1.7	0.06
12	1.4	0.05
16	1.0	0.03
--	µm	±µm
22	710	25
25	600	21
30	500	18
36	425	15
44	355	13
60	250	13(9.9) **
85	180	11(7.6)
100	150	9.4(6.6)
120	125	8.1(5.8)
150	106	7.4(5.2)
170	90	6.6(4.6)
200	75	6.1(4.1)
240	63	5.3(3.7)
300	53	4.8(3.4)
350	45	4.8(3.1)

* Sieve number is the number of meshes in a length of 2.24 cm. In each transverse direction parallel to the wires.
** Figures in brackets refer to close tolerances, those without brackets relate to full tolerances.

1.3 Thermometers

Unless otherwise specified, thermometers suitable for pharmacopoeial tests conform to Indian Standard 4825-1968 and are standardised in accordance with the `Indian Standard Method of Calibrating Liquid-in-Glass Thermometers', 6274-1971.

The thermometers are of the mercury-in-glass type and are filled with a dried inert gas, preferably nitrogen. They may be standardised for total immersion or for partial immersion. Each thermometer should be employed according to the condition of immersion under which it was standardised. In the selection of the theremometer it is essential to consider the conditions under which it is to be used.

1.4 Volumetric Glassware

Volumetric apparatus is normally calibrated at 27º. However, the temperature generally specified for measurements of volume in the analytical operations of the pharmacopoeia, unless otherwise stated, is 25º. The discrepancy is inconsequential as long as the room temperature in the laboratory is reasonably constant and is around 27º.

Pharmacopoeial assays involving volumetric measurements require the use of accurately calibrated glassware. Volumetirc apparatus must be suitably designed to assure accuracy. The design, construction and capacity of volumetric glassware should be in accordance with those laid down by the Indian Standards Institution. The tolerances on capacity for volumetric flasks, pipettes and burettes, as laid down in the relevant Indian Standards, are set out in the following table.

Volumetric Flask : I.S. 915-1975

Nominal capacity, ml	5	10	25	50	100	250	500	1000
Tolerance, ± ml	0.02	0.02	0.03	0.04	0.06	0.1	0.15	0.2

One Mark Pipettes : I.S. 1117 -1975

Nominal capacity, ml	1	2	5	10	20	25	50	100
Tolerance, ± ml	0.01	0.01	0.02	0.02	0.03	0.03	0.04	0.06

Graduated Pipettes : I.S. 4162-1967

Nominal capacity, ml	1	2	5	10	25
Subdivision, ml	0.01	0.02	0.05	0.10	0.2
Tolerance, ± ml	0.006	0.01	0.03	0.05	0.1

Burettes : I.S. 1997 – 1967

Nominal capacity, ml	10	25	50	10
Subdivision, ml	0.05	0.05	0.1	0.1
Tolerance, ± ml	0.01	0.03	0.05	0.1

1.1.5 Weights and Balances

Pharmacopoeial tests and assays require the use of analytical balances that vary in capacity, sensitivity and reproducibility. The accuracy needed for a weighing should dictate the type of balance. Where substances are to be "accurately weighed", the weighing is to be performed so as to limit the error to not more than 0.1 per cent. For example, a quantity of 50 mg is to be weighed to the nearest 0.05 mg; a quantity of 0.1 g is to be weighed to the nearest 0.1 mg; and quantity of 10 g is to be weighed to the nearest 10 mg. A balance should be chosen such that the value of three times the standard deviation of the reproducibility of the balance, divided by the amount to be weighed, does not exceed 0.001.

APPENDIX-2

2.1 TESTING OF DRUGS

2.1.1.-Systematic Study of Crude Drugs

In the Indian Systems of Medicine comprising of Ayurveda, Unani and Siddha, drugs of plant, animal and mineral origin, are used in their natural or so called "Crude" forms singly or in their mixture or in combination, to make a compound preparation of formulation. Nearly 90 per cent of the Crude Drugs are obtained from the plant sources while about 10 per cent of the drugs are derived from animal and mineral sources. The drugs of plant origin especially of herbaceous nature are frequently used as whole plant; otherwise their parts such as Root, Stem, Leaf, Flower, Seed, Fruit modifications of Stem and Root, Bark of a Stem or Root, Wood, and their Exudates or Gums etc. constitute single drugs in the Indian Systems of Medicine. These vegetable drugs are either used in dried forms or some times as whole fresh or their juice. The study of these crude drugs made with a view to recognise them is called Pharmacognosy (Pharmakon = Drug; Gignosco = to acquire knowledge of), meaning the knowledge or science of Drugs. In Pharmacognosy a complete and systematic study of a drug is done, which comprises of (I) origin, common names, scientific nomenclature and family, (ii) geographical source (and history), (iii) cultivation, collection, preservation and storage, (iv) Macroscopical, Microscopical and sensory (organoleptic) characters, (v) Chemical composition wherever possible, (vi) Identity, Purity, Strength and Assay, (vii) substitute and adulterants etc. Such systematic study of a drug as complete as possible, is claimed to be the scientific or pharmacognositical evaluation.

As mentioned above each crude drug derived from the vegetable kingdom consists of a definite part of plant e.g., leaf, stem, fruit, seed, wood, bark, root etc. Morphological or Macroscopical details of the respective part are given by observing it with a naked eye or with the aid of a magnifying lens. In this description general conditions of the drug, size, shape, outer surface, inner surface etc are referred to. Drugs can be identified with the aid of the above, only if they are available in entire condition. Sensory or Organoleptic characters describe colour, odour, taste, consistency etc. The microscopic examination of different parts of the drug provides several diagnostic characters. In case of leaves, surface preparation and transverse section, preferably through midrib, are made and nature of epidermis, trichomes, stomata, arrangement of tissues like palisade cells, vascular bundles and nature of cell content are studied. Similarly in case of bark, root, rhizome and wood, transverse and longitudinal sections are made and from characteristic arrangements of tissues of each drug and from diagnostic elements like stone cells, fibres, vessels etc. as also from the study of the cell deposits like crystals, starch etc., the drugs are identified. The studies of diagnostic elements are helpful especially when the drugs are in powdered condition and give clues in the identification of drugs. Linear measurements and other methods of quantitative microscopy give further aid in the identification of the drugs. The sections or the powdered drugs samples are cleared by clearing agents, mostly chloral-hydrate solution, before mounting on the slide.

The basic chemical nature of cell-wall of almost all the plants is cellulosic, However, lignin, suberin, cutin or mucilage are deposited on the cellulose. Cellulose gives blue colour with chlorzinc-iodine solution or with cuoxam. (Copper-oxide-ammonia) reagent. Lignin present in the middle lamella and secondary cell-well of many vessels, fibres and sclerieds gives red colour with phloroglucinol and concentrated hydrochloric acid. Suberin is present in cork and endodermis cells while cutin in the cuticle of leaf. Both are fatty in nature and when heated with Sudan Red-III give red colour.

Mucilage gives red colour with ruthenium red. The chemical constituents present in the drugs can be identified by chemical or microchemical tests e.g., Rhubarb rhizomes give with 5% potassium hydroxide red colour because of anthraquinone derivatives, strychnine present in Nux-vomica gives purplish-red colour with ammonium vanadate and concentrated sulphuric acid.

Paper and Thin Layer Chromotography are now utilised in identification of drugs, their adulterant and their chemical constituents. Methods have been developed for quantitative estimation of the chemical constituents from Paper and Thin Layer Chromatography (TLC).

2.1.2. –Microscopical Methods of Examining Crude Vegetable Drugs

Methods of preparing specimens of crude materials of vegetable drugs for microscopical studies vary, depending on the morphological groups of drugs to be examined and also on the natures of the material i.e., entire, cut or powdered.

I. LEAVES, HERBS AND FLOWERS

For examining leaves, herbs and flowers (entire or cut) under microscope, following methods are employed for clarification :

A. Entire and cut materials

(i) ***Entire materials –*** When examining entire leaves, herbs and flowers, take pieces of leaf (margin and vein of leaves only), herbs (only leaf) and flowers (only calyx and corolla) in test tube. Add a solution of caustic alkali or nitric acid to the test tube and boil for 1-2 minutes, pour the contents into a porcelain dish, drain off the liquid, wash the material with water and leave for sometimes. Remove the pieces of the material from the water with a spatula and put on the slide, add a few drops of the solution of *glycerol or chloral hydrate.* Crush the material with scalpel and cover with cover slip before examining.

(ii) ***Cut materials –***For examining cut leaves, herbs and flowers, take several pieces in a test tube and employ the same methods as described for entire materials.

Other methods employed for clarification of the material (leaf and stem) are described below :-

(a) Leaf –Boil pieces of leaves in a test tube with chloral hydrate for several minutes until completely clarified and then examine them in chloral hydrate solution. After clarification, leaf pieces are divided into two parts with the help of a scalpel or needle, and carefully turn one part. The leaf can be examined from both the dorsal and ventral surfaces.

(b) Stem –To examine stem material (without leaf) boil pieces in a solution of *caustic alkali* or in *nitric acid.* Remove the epidermis with a scalpel or a needle for examining the surface. For examining pressed specimen of stem, take separate tissue and press them with a scalpel on the slide.

B. Powder

For examining characters of the powder take sufficient amount of powder in Chloral-hydrate solution on a slide and cover it with a cover slip, warm over a low flame for a short time.

II. FRUITS AND SEEDS

A. Entire materials

For microscopical examination of fruit and seed take the specimens or outer coat of seed or fruit and examine as described below :

(i) ***Outer Coat –***For examining the outer coat boil 3 or 4 seeds or fruits in caustic alkali solution in a test tube for 1-2 minutes (outer coat specimens with intensive pigmentation are boiled for longer period). After boiling, place the pieces on slide, remove the layers of the coat and examine them after mounting in glycerol solution.

(ii) ***Section –***If fruits or seeds are too hard to cut then boil them for 15-30 minutes or more depending on their hardness or keep them in moistening chamber or absorb in water and chloroform solution or soften them with stem and then cut the specimen for examining purpose. For cutting small, flat seeds (which are difficult to hold) place them in a pith or potato slit for section cutting. Small, round or

smooth seeds cannot be cut into section in the pith, then in such cases, they may be embedded in paraffin wax blocks for section cutting. For this, a block of paraffin (0.6 × 0.5 × 1.5 cms. in size) is made and the seed is embedded in the block by making a cavity or a pit in the block with a hot teasing needle. Cut the section with a sharp razor (through the object) together with the paraffin, place them on to the slide, remove paraffin with a needle or wash it with xylene and examine the section in *chloral-hydrate solution.*

B. Powder

For examining the structure of the cells of the seed coat and the cells of the embryo take a small amount of powder of the material on a slide in glycerol and cover it with a cover slip and examine.

1. *Starch* – For examining the presence of starch in the seed, take two specimens, one in iodine solution and the other in water. With iodine solution starch turns blue. Shape and the structure of starch grains can be seen in water and their size is measured.

When examining objects containing starch, prepare specimen by slightly warming in chloral-hydrate solution.

2. *Fixed Oil* – For examining the presence of fixed oil, prepare a specimen in a solution of Sudan III droplets of fixed oil are coloured orange pink. When examining objects containing small amount of fixed oil, prepare a specimen by slightly warming in chloral-hydrate solution, and when examining objects containing large amount of fixed oil, then the powder is de-fatted and clarified as follows :

Place 0.5 g. of the powder in a porcelain dish, add 5-10 ml. of dilute nitric acid and boil for 1 minute, then strain off the liquid through a cloth, wash the residue with hot water and return it to the porcelain dish with a spatula, boil it with 5-10 ml of *caustic alkali solution* for 1 minute and again strain it through the cloth and wash with water. Examine the residue in a glycerol solution, after the treatment the structure of the layers of the coat and their cells can be seen very distinctly.

3. *Mucilage* –Prepare a specimen in Ruthenium Red and examine it under a low power microscope or under dissecting microscope. Mucilage appears as pinkish-red or yellow coloured masses.

III. BARKS

A. Entire material

Prepare transverse or longitudinal section of bark. To soften bark break it into pieces of about 1-2 cm long and 0.5-1 cm wide and boil with in a test tube for 1-3 minutes. Soft pieces are then straightened with a scalpel so as to have a exact transverse or longitudinal direction. Cut the section with razor, moisten the surface of the bark with glycerol solution. Remove the sections with a brush and place them on the slide. Thin pieces of the bark are cut by placing them in the pith (potato or carrot). The sections are treated with various reagents before examining.

1. *Lignified elements* –For testing lignin add several drops of *phloroglucinol* and a drop of *concentrated hydrochloric acid* to the section on a slide then draw off the liquid, immerse the section in *chloral hydrate solution* and cover with a cover slip (the specimen should not be heated); the lignified elements are coloured crimson. *Phloroglucinol* can be substituted by *saffranine*, and the lignified elements are coloured pink. The excessive stain can be washed out with acidified alcohol.

2. *Starch* – Starch is detected by treating with iodine solution.

3. *Tannin* –Tannin is detected by treating with *ferric ammonium sulphate solution* (blue-black or green black colour shows the presence of Tannin) or with *potassium-bi-chromate solution* (brown colour indicates the presence of Tannin).

4. ***Anthraquinone derivatives –***Anthraquinone derivatives are detected by treating with alkali solution (blood-red colour shows the presence of anthraquinone derivatives).

B. Cut materials

Prepare small pieces or scraping of bark and boil them for 3-5 minutes in a solution of *caustic alkali* or *potassium hydroxide* or in *nitric acid solution* and then mount in *glycerin* for examination on a slide covered with a cover slip.

C. Powder

Prepare specimen for examination by placing a little amount of powder on a slide, add 1-2 drops of *phloroglucinol* and a drop of concentrated *hydrochloric acid*, cover it with a cover slip, draw off the liquid from one side of the slide with filter paper, and then apply 1-2 drops of *chloral-hydrate solution* from the other side of the slide, lignified elements are stained crimson-red. Specimen may also be prepared with *caustic alkali or ferric ammonium sulphate* for this purpose.

IV. ROOTS AND RHIZOMES

A. Entire materials

For anatomical examination of entire roots and rhizomes cut transverse and longitudinal sections. For this, soften small pieces of roots without heating in *glycerol solution* for 1-3 days, depending on their hardness. The softened roots are straightened with the help of a scalpel in the right direction and then cut a section with the razor. First, cut thicker entire slices and then make thin, smaller sections. Stain the entire slices with *phloroglucinol* and *concentrated hydrochloric acid* or with *safranin* examine the specimen under a dissecting microscope. For micro-chemical test the small and thin sections are examined under microscope, as follows :

1. ***Starch –*** Starch is detected with iodine solution. For this, prepare specimen with water to measure the granule of starch with an occular micrometer.

2. ***Inulin –***Inulin is detected with Molish's reagent. For this place a little powder on a slide and apply 1-2 drops of naphthol and a drop of concentrated sulphuric acid, if inulin is present, the powder will appear reddish-violet coloured. Starch also gives this test, so the test for inulin can be done in the absence of starch.

3. ***Lignified elements –***Lignified elements (fibrovascular bundles, mechanical tissue etc.) are detected with *phloroglucinol* and concentrated *hydrochloric acid* or *safranine solution* as mentioned above for barks.

4. ***Fixed oil –***For fixed oil detection use Sudan IV, as mentioned above for fruits and seeds.

If required for tannin, anthraquinone derivatives, test as mentioned above.

B. Cut material

Make small pieces or scrapping of roots or rhizomes and boil them for 3-5 minutes in *caustic alkali,* or in *nitric acid* and then make pressed specimen and immerse them in *glycerol.*

Microchemical tests can be performed with scrapings for various chemicals as mentioned above.

C. Powder

Prepare several specimens of the powder on slides in *chloral hydrate solution* and perform the above mentioned standard tests for detection of starch, fixed oil, inulin, lignified elements, anthraquinone derivatives, tannins, mucilage, etc.

2.1.3. –Types of Stomata

There are several types of stomata, distinguished by the form and arrangement of the surrounding cells. The following descriptions apply to mature stomata.

1. Anomocytic (irregular-celled) –Previously known as ranunculaceous. The stoma is surrounded by a varying number of cells in no way differing form those of the epidermis generally.

2. Anisocytic (unequal-celled) –Previously known as cruciferous or solanaceous. The stoma is usually surrounded by three subsidiary cells, of which one is markedly smaller than the others.

3. Diacytic (cross-celled) –previously known as caryophyllaceous. The stoma is accompanied by two subsidiary cells whose common wall is at right angles to the guard cells.

4. Paracytic (parallel-celled) –Previously known as rubiaceous. The stoma has one each side one or more subsidiary cells parallel to the long axis of the pore and guard cells.

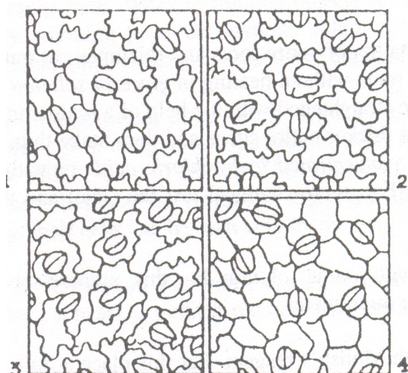


Fig. 1 Various types of stomata

2.1.4 – Determination of Stomatal Index

The stomatal index is the percentage of the number of stomata formed by the total number of epidermal cells, including the stomata, each stoma being counted as one cell.

Place leaf fragments of about 5 × 5 mm in size in a test tube containing about 5 ml of *chloral hydrate solution* and heat in a boiling water-bath for about 15 minutes or until the fragments become transparent. Transfer a fragment to a microscopic slide and prepare the mount, the lower epidermis uppermost, in chloral hydrate solution and put a small drop of glycerol-ethanol solution on one side of the cover-glass to prevent the preparation from drying. Examine with a 40x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Mark on the drawing paper a cross (x) for each epidermal cell and a circle (o) for each stoma. Calculate the result as follows :

$$\text{Stomatal index} = \frac{S \times 100}{E + S}$$

Where S = the number of stomata in a given area of leaf ; and
E = the number of epidermal cells (including trichomes) in the same area of leaf.

For each sample of leaf make not fewer than ten determinations and calculate the average index.

2.1.5. – Determination of Palisade Ratio

Palisade ratio is the average number of palisade cells under one epidermal cell.

Place leaf fragments of about 5 × 5 mm in size in a test-tube containing about 5 ml of chloral hydrate solution and heat in a boiling water-bath for about 15 minutes or until the fragments become transparent. Transfer a fragment to a microscopical slide and prepare the mount of the upper epidermis in chloral hydrate solution and put a small drop of glycerol solution on one side of the cover-glass to prevent the preparation from drying. Examine with a 40x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Trace four adjacent epidermal cells on paper; focus gently downward to bring the palisade into view and trace sufficient palisade cells to cover the area of the outlines of the four epidermal cells. Count the palisade cells under the four epidermal cells. Where a cell is intersected, include it in the count only when more than half of it is within the area of the epidermal cells. Calculate the average number of palisade cells beneath one epidermal cell, dividing the count by 4; this is the "Palisade ratio" (See Fig. 2).

For each sample of leaf make not fewer than ten determinations and calculate the average number.

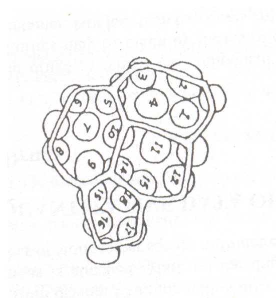


Fig. 2 Palisade ratio $\frac{18.4}{4} = 4.5$

2.1.6 –Determination of Vein-Islet Number

The mesophyll of a leaf is divided into small portions of photosynthetic tissue by anastomosis of the veins and veinlets; such small portions or areas are termed "Vein-Islets". The number of vein-islets per square millimeter is termed the "Vein-Islet number". This value has been shown to be constant for any given species and, for full-grown leaves, to be unaffected by the age of the plant or the size of the leaves. The vein-islet number has proved useful for the critical distinction of certain nearly related species. The determination is carried out as follows :

For Whole or Cut leaves —Take pieces of leaf lamina with an area of not less than 4 square millimeters from the central portion of the lamina and excluding the midrib and the margin of the leaf. Clear the pieces of lamina by heating in a test tube containing c*hloral hydrate solution* on a boiling water-bath for 30 to 60 minutes or until clear and prepare a mount in *glycerol-solution* or, if desired, stain with *safranin solution* and prepare the mount in *Canada Balsam.* Place the stage micrometer on the microscope stage and examine with 4x objective and a 6x eye piece. Draw a line representing 2 mm on a sheet of paper by means of a microscopical drawing apparatus and construct a square on the line representing an area of 4 square millimeters. Move the paper so that the square is seen in the centre of the field of the eyepiece. Place the slide with the cleared leaf piece on the microscope stage and draw in the veins and veinlets included within the square, completing the outlines of those vein-islets which overlap two adjacent sides of the square. Count the number of vein-islets within the square including those overlapping on two adjacent sides and excluding those intersected by the other two sides. The result obtained is the number of vein-islets in 4 square millimeters. For each sample of leaf make not fewer than three determinations and calculate the average number of vein-islets per square millimeter.

For Leaf Fragments having an area less than 4 square millimeters – Take fragments of leaf lamina each with an area of not less than 1 square millimeter, excluding the midrib and the margin of the leaf. Clear and prepare a mount as stated above. Use a 10x objective and a 6x eyepiece and draw a line representing 1 mm on a sheet of paper by means of a microscopial drawing apparatus and construct a square on this line representing an area of 1 square millimetre. Carry out the rest of the procedure as stated above. The result obtained is the number of vein-islets in 1 square millimetre. For each sample of leaf make no less than 12 determinations and calculate the average number.

2.1.7 Determination of Stomatal Number

Place leaf fragments of about 5x5 mm in size in a test tube containing about 5 ml of chloral hydrate solution and heat in a boiling water-bath for about 15 minutes or until the fragments become transparent. Transfer a fragments to a microscopic slide and prepare the mount the lower epidermis uppermost, in chloral hydrate solution and put a small drop of glycerol-ethanol solution on one side of the cover glass to prevent the preparation from drying. Examine with a 40 x objective and a 6x eye piece, to which a microscopical drawing apparatus is attached. Mark on the drawing paper a cross (x) for each stomata and calculate the average number of stomata per square millimetre for each surface of the leaf.

2.2. DETERMINATION OF QUANTITATIVE DATA OF VEGETABLE DRUGS

2.2.1 – Sampling of Vegetable Drugs

Original Samples

(a) Samples of crude vegetable drugs in which the component parts are 1 cm or less in any dimension; and of powdered or ground drugs may be taken by means of sampling device that removes a core from the top to the bottom of the container. Not less than two cores are taken in opposite directions.

When the total weight of the drug to be sampled is less than 100 Kg, at least 250 g are withdrawn to constitute an original sample.

When the total weight of the drug to be sampled is more than 100 Kg, several samples are taken in the manner described, mixed and quartered, two of the diagonal quarters being rejected, and the remaining two quarters being combined and carefully mixed, and again subjected to a quartering process in the same manner until each of the quarters weigh at least 125 g; two such quarters then constitute an original sample.

(b) Samples of crude vegetable drugs in which the component parts are over 1 cm in any dimension may be taken by hand.

When the total weight of the drug to be sampled is less than 100 Kg, samples are taken from different parts of the container or containers. Not less than 500 g of samples so taken constitute an original sample.

When the total weight of the drug to be sampled is more than 100 Kg, several samples are taken in the manner described, mixed and quartered, two of the diagonal quarters being rejected, and the remaining two quarters being combined and carefully mixed, and again subjected to a quartering process in the same manner until each of the quarters weigh not less than 250 g; two such quarters then constitute an original sample.

NOTE :- Where the total weight of crude drug to be sampled is less than 10 Kg, the preceding methods may be followed but somewhat smaller quantities are to be withdrawn but in no case shall the original samples weight less than 125 g.

Test sample

Withdraw as much as may be necessary of the original sample by quartering, taking care to see that the portion is representative of the gross sample. In the case of unground or unpowdered drugs, grind the sample so that it will pass through a No. 22 sieve. If the sample cannot be ground, it should be reduced to as fine a state as possible. Mix by rolling it in paper or cloth, spread it out in a thin layer, and withdraw the portion for analysis.

2.2.2 –Foreign Matter and Determination of Foreign Matter

A. FOREIGN MATTER

Drugs should be free from moulds, insects, animal faecal matter and other contaminations such as earth, stones and extraneous material.

Foreign matter is material consisting of any or all of the following :-

(1) In particular, parts of the organ or organs from which the drug is derived other than the parts named in the definition or for which a limit is prescribed in the individual monograph.

(2) Any organ or part of organ, other than those named in the definition and description.

The amount of foreign matter shall not be more than the percentage prescribed in the monograph.

B. DETERMINATION OF FOREIGN MATTER

Weigh 100 –500 g of the drug sample to be examined, or the minimum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (6x). Separate and weigh it and calculate the percentage present .

2.2.3. –Determination of Total Ash

Incinerate about 2 to 3 g accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450º until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450º. Calculate the percentage of ash with reference to the air-dried drug.

2.2.4. –Determination of Acid Insoluble Ash

Boil the ash obtained in (2.2.3) for 5 minutes with 25 ml of *dilute hydrochloric acid;* collect the insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

2.2.5. –Determination of Water Soluble Ash

Boil the ash for 5 minutes with 25 ml of water; collect insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot water, and ignite for 15 minutes at a temprature not exceeding 450º. Substract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

2.2.6. –Determination of Alcohol Soluble Extractive

Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of Alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105º, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

2.2.7. –Determination of Water Soluble Extractive

Proceed as directed for the determination of Alcohol-soluble extractive, using *chloroform water* instead of ethanol.

2.2.8. –Determination of Ether Soluble Extractive (Fixed Oil Content)

Transfer a suitably weighed quantity (depending on the fixed oil content) of the air dried, crushed drug to an extraction thimble, extract with *Solvent ether* (or petroleum *ether,* b.p. 40º to 60º) in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. Filter the extract quantitatively into a tared evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105º to constant weight. Calculate the percentage of ether-soluble extractive with reference to the air-dried drug.

2.2.9. –Determination of Moisture Content (Loss on Drying)

Procedure set forth here determines the amount of volatile matter (i.e., water drying off from the drug). For substances appearing to contain water as the only volatile constituent, the procedure given below, is appropriately used.

Place about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tared evaporating dish. For example, for underground or unpowderdd drug, prepare about 10 g of the sample by cutting shredding so that the parts are about 3 mm in thickness.

Seeds and fruits, smaller than 3 mm should be cracked. Avoid the use of high speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. After placing the above said amount of the drug in the tared evaporating dish dry at 105º for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighings corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighings after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference.

2.2.10. –Determination of Volatile Oil in Drugs

The determination of volatile oil in a drug is made by distilling the drug with a mixture of *water* and *glycerin,* collecting the distillate in a graduated tube in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask, and measuring the volume of the oil. The content of the volatile oil is expressed as a percentage v/w.

The apparatus consists of the following parts (See Fig. 3) . The apparatus described below is recommended but any similar apparatus may be used provided that it permits complete distillation of the volatile oil. All glass parts of the apparatus should be made of good quality resistance glass.

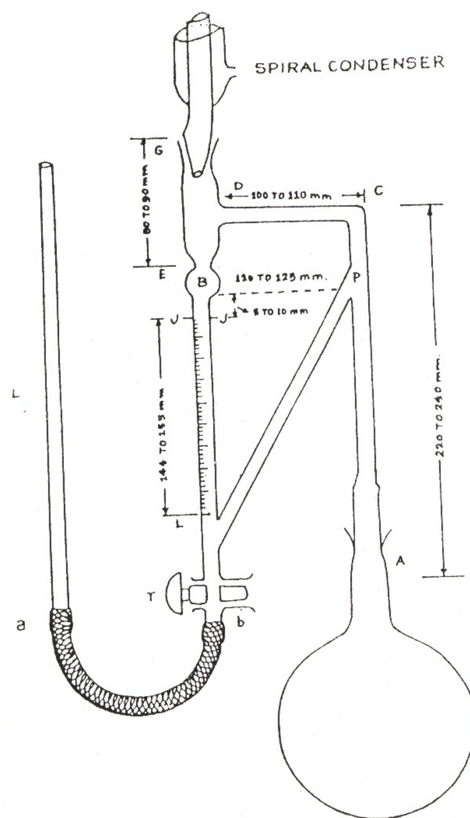


Fig. 3 Apparatus for volatile oil determination

(a) Distilling Flask –A spherical flask, 1,000 ml capacity with ground neck, taper of ground socket 1 in 10, internal diameter of larger end 34.35 to 34.65 mm

(b) Still head –graduated measuring tube, and return flow tube made in one piece, in accordance with the following specifications. External diameter of the smaller end 31.0 to 31.2 mm. Minimum length of the ground zone –34 mm.

Tube AC, length –220 to 240 mm.
Internal diameter –13 to 15 mm.

Bulb CD, length –100 to 110 mm.
Internal diameter –13 to 15 mm.

Spiral condenser –ground joint accurately fitting in the ground neck of the tube EG, taper 1 in 10.

Tube EG, length –80 to 90 mm.
Internal Diameter –30 to 40 mm.
Bulb B –length 20 to 22 mm.
Internal diameter –15 to 20 mm.

The distance between B and P is 120 to 125 mm.

Junction P and the centre of the bulb B must be in the same horizontal plane.

Measuring tube JL –length of the graduated portion 144 to 155 mm capacity 2 millilitres graduated into fifths and fiftieths of a millilitre.

Tube PL –return flow tube –Internal diameter –7 to 8 mm.
Levelling tube I, length –450 to 500 mm. Internal diameter 10 to 12 mm tapering at the lower end with a wide top (20 to 25 mm diameter).
Rubber tubing a—b length 450 to 500 mm. Internal diameter 5 to 8 mm.

(c) Burner – A luminous Argand burner with chimney and sensitive regulative tap.

(d) Stand –A retort stand with asbestos covered ring and clamp carrying a piece of metal tubing connected by a short length of rubber tubing with the water inlet tube of the condenser jacket.

The Whole of the apparatus is effectively screened from draught.

The apparatus is cleaned before each distillation by washing successively with *acetone* and *water,* then inverting it, filling it with *chromic sulphuric acid* mixture, after closing the open end at G, and allowing to stand, and finally rinsing with water.

Method of determination

A suitable quantity of the coarsely powdered drug together with 75 ml of *glycerin* and 175 ml of *water* in the one litre distilling flask, and a few pieces of porous earthen ware and one filter paper 15 cm cut into small strips, 7 to 12 mm wide, are also put in the distilling flask, which is then connected to the still head. Before attaching the condenser, water is run into the graduated receiver, keeping the tap T open until the water overflows, at P. Any air bubbles in the rubber tubing a—b are carefully removed by pressing the tube. The tap is then closed and the condenser attached. The contents of the flask are now heated and stirred by frequent agitation until ebullition commences. The distillation is continued at a rate which keeps the lower end of the condenser cool. The flask is rotated occasionally to wash down any material that adheres to its sides.

At the end of the specified time (3 to 4 hours) heating is discontinued, the apparatus is allowed to cool for 10 minutes and the tap T is opened and the tube L_1 lowered slowly; as soon as the layer of the oil completely enters into the graduated part of the receiver the tap is closed and the volume is read.

The tube L_1 is then raised till the level of water in it is above the level of B, when the tap T is slowly opened to return the oil to the bulb. The distillation is again continued for another hour and the volume of oil is again read, after cooling the apparatus as before. If necessary, the distillation is again continued until successive readings of the volatile oil do not differ.

The measured yield of volatile oil is taken to be the content of volatile oil in the drug.

The dimensions of the apparatus may be suitably modified in case of necessity.

TABLE

Drug	Weight to be taken	Condition When distilled	Approximate time of distillation in houts
1	2	3	4
Ajamōdā	20	Whole	4
Dhānyaka	40	No.10 powder	3
Guggulu	40	Whole	3

Haridrā	20	No. 20 powder	5
Jatāmānsi	20	Whole	4
Jatiphala	15	No. 20 powder	3
Kṛṣṇajīraka	20	Whole	4
Lavaṅga	4	Coarsely crushed	4
Miśrēya	25	Whole	4
Sukṣmaila	20	Seed only, Whole	5
Tvakpatra	40	No.10 powder	5
Tvak	40	No.10 powder	5
Yavāni	20	Whole	4

2.2.11. –Special processes used in Alkaloidal Assays

2.2.11.a –CONTINUOUS EXTRACTION OF DRUG –

Where continuous extraction of a drug of any other substance is recommended in the monograph, the process consists of percolating it with a suitable solvents at a temperature approximately that of the boiling point of the solvent. Any apparatus that permits the uniform percolation of the drug and the continuous flow of the vapour of the solvent around the percolator may be used. The type commonly known as the Soxhlet apparatus is suitable for this purpose.

A simple apparatus is shown in the accompanying illustraion. A is an outer tube of stout glass; the wider part is about 18 cm in length and has an internal diameter of 4.8 to 5 cm; the lower end C is about 5 cm in length and has an external diameter of about 1.6 cm . B is a straight glass tube open at both ends, about 9 cm in length and having an external diameter of about 3.8 cm; over its lower flanged end is tied firmly with a piece of calico or other suitable material. D is a glass coil, which supports the margin of the tube B and prevents it from resting in contact with the outer tube A. The lower end C of the outer tube A is fitted by a cork to the distilling flask E, in which a suitable quantity of the solvent has been placed. The substance to be extracted, previously moistened with the solvent or subjected to any preliminary treatment required, is introduced into the inner tube B, which is supported so that the percolate drops into the outer tube. A pad of cotton wool G is placed on the top of the drug, the inner tube is lowered into position and the outer tube connected by means of a suitable cork with the tube of a reflux condenser F. The flask is heated and the extraction continued as directed (See Fig. 4).

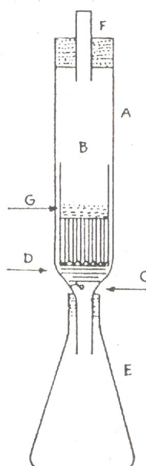


Fig. 4 Apparatus for the continuous extraction of Drugs

2.2.11.b –TESTS FOR COMPLETE EXTRACTION OF ALKALOIDS–Complete extraction is indicated by the following tests :

When extracting with an aqueous or alcoholic liquid –After extracting at least three times with the liquid, add to a few drops of the next portion, after acidifying with 2 *N hydrochloric acid* if necessary, 0.05 ml of potassium mercuri-iodide solution or for solanaceous alkaloids 0.05 ml of *potassium iodobismuthate solution;* no precipitate or turbidity, is produced.

When extracting with an immiscible solvent –After extracting at least three times with the solvent, add to 1 to 2 ml of the next portion 1 to 2 ml of 0.1 *N hydrochloric acid,* remove the organic solvent by evaporation, transfer the aqueous residue to a test tube, and add 0.05 ml of *potassium mercuri-iodide solution* for solanaceous alkaloids 0.05 ml of *potassium iodobismuthate solution* or for emetine, 0.05 ml of *iodine solution;* not more than a very faint opalescenece is produced.

2.3. LIMIT TESTS

2.3.1 Limit Test for Arsenic

In the limit test for arsenic, the amount of arsenic present is expressed as arsenic, As

Apparatus –

A wide-mouthed bottle capable of holding about 120 ml is fitted with a rubber bung through which passes a glass tube. The latter, made from ordinary glass tubing, has a total length of 200 mm and an internal diameter of exactly 6.5 mm (external diameter about 8 mm). It is drawn out at one end to a diameter of about 1 mm and a hole not less than 2 mm in diameter is blown in the side of the tube, near the constricted part. When the bung is inserted in the bottle containing 70 ml of liquid, the constricted end of the tube is above the surface of the liquid, and the hole in the side is below the bottom of the bung. The upper end of the tube is cut off square, and is either slightly rounded or ground smooth.

Two rubber bungs (about 25 mm X 25 mm), each with a hole bored centrally and true, exactly 6.5 mm in diameter, are fitted with a rubber band or spring clip for holding them tightly together. Alternatively the two bungs may be replaced by any suitable contrivance satisfying the conditions described under *the General Test.*

Reagents –

Ammonium oxalate AsT : *Ammonium oxalate* which complies with the following additional test :

Heat 5 g with 15 ml of *water,* 5 ml of *nitric acid AsT,* and 10 ml of *Sulphuric acid AsT* in narrow necked, round-bottomed flask until frothing ceases, cool, and apply the General Test; no visible stain is produced.

Arsenic solution, dilute, AsT :

Strong Arsenic solution AsT		1 ml
Water	sufficient to produce	100 ml

Dilute arsenic solution AsT must be freshly prepared.
1 ml contains 0.01 mg of arsenic, As.

Arsenic solution, strong, AsT :

Arsenic trioxide		0.132 g
Hydrochloric acid		50 ml
Water	sufficient to produce	100 ml

Brominated hydrochloric acid AsT :

Bromine solution AsT	1 ml
Hydrochloric acid AsT	100 ml

Bromine solution AsT :

Bromine		30 g
Potassium bromide		30 g
Water	sufficient to produce	100 ml

It complies with the following test :

Evaporate 10 ml on a water-bath nearly to dryness, add 50 ml of water, 10 ml of *hydrochloric acid AsT* and sufficient *stannous chloride solution AsT* to reduce the remaining bromine and apply the General Test; the stain produced is not deeper than 1 ml *standard stain,* showing that the proportion of arsenic present does not exceed 1 part per million.

Citric acid AsT : *Citric acid* which complies with the following additional tests : Dissolve 10 g in 50 ml of water add 10 ml of *stannated hydrochloric acid AsT* and apply the General Test; no visible stain is produced.

Hydrochloric acid AsT : *Hydrochloric acid* diluted with *water* to contain about 32 per cent w/w of HCI and complying with the following additional tests :

(i) Dilute 10 ml with sufficient water to produce 50 ml, add 5 ml of *ammonium thiocyanate solution* and stir immediately; no colour is produced.

(ii) To 50 ml add 0.2 ml of *bromine solution AsT,* evaporate on a water-bath until reduced to 16 ml adding more *bromine solution AsT,* if necessary, in order that an excess, as indicated by the colour, may be present throughout the evaporation; add 50 ml of *water* and 5 drops of *stannons chloride solution AsT,* and apply the General Test; the stain producted is not deeper than a 0.2 ml *standard stain* prepared with the same acid, showing that the proportion of arsenic present does not exceed 0.05 part per million.

Hydrochloric acid (constant-boiling composition) AsT : Boil *hydrochloric acid AsT to constant boiling Composition* in the presence of *hydrazine hydrate*, using 1 ml of 10 per cent w/v solution in *water* per litre of the acid.

Mercuric chloride paper – Smooth white filter paper, not less than 25 mm in width, soaked in a saturated solution of *mercuric chloride,* pressed to remove superfluous solution, and dried at about 60º, in the dark. The grade of the filter paper is such that the weight is between 65 and 120 g per sq. mm; the thickness in mm of 400 papers is approximately equal numerically, to the weight in g per sq. mm.
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NOTE –murcuric chloride paper should be stored in a stoppered bottle in the dark. Paper which has been exposed to sunlight or to the vapour of ammonia affords a lighter stain or no stain at all when empolyed in the limit test for arsenic.

Nitric acid AsT : *Nitric acid* which complies with the following additional test :

Heat 20 ml in a porcelain dish with 2 ml of *sulphuric acid AsT,* until white fumes are given off. Cool, add 2 ml of water, and again heat until white fumes are given off; cool, add 50 ml of water and 10 ml of *stannated hydrochloric acid AsT,* and apply the General Test; no visible stain is produced.

Potassium chlorate AsT : *Potassium chlorate* which complies with the following additional test :

Mix 5 g in the cold with 20 ml of *water* and 22 ml of *hydrochloric acid AsT;* when the first reaction has subsided, heat gently to expel chlorine, remove the last traces with a few drops of *stannous chloride solution AsT,* add 20 ml of water, and apply the General Test; no visible stain is produced.

Potassium iodide AsT : *Potassium iodide* which complies with the following additional test :

Dissolve 10 g in 25 ml of *hydrochloric acid AsT* and 35 ml of *water,* add 2 drops of *stannous chloride solution AsT* and apply the General Test; no visible stain is produced.

Sodium carbonate, anhydrous AsT : *Anhydrous sodium carbonate* which complies with the following additional test :

Dissolve 5 g in 50 ml of *water,* add 20 ml of *brominated hydrochloric acid AsT,* remove the excess of bromine with a few drops of *stannous chloride solution AsT,* and apply the General Test; no visible stain is produced.

Stannated hydrochloric acid AsT :

Stannous chloride solution AsT	1ml
Hydrochloric Acid AsT	100 ml

Stannous chloride solution AsT : Prepared from *stannous chloride solution* by adding an equal volume of *hydrochloric acid*, boiling down to the original volume, and filtering through a fine-grain filter paper.

It complies with the following test :

To 10 ml add 6 ml of water and 10 ml of *hydrochloric acid AsT,* distil and collect 16 ml. To the distillate and 50 ml of *water* and 2 drops of *stannuous chloride solution AsT* and apply the General Test; the stain produced is not deeper than a 1-ml *standard stain,* showing that the proportion of arsenic present does not exceed 1 part per million.

Sulphuric acid AsT : *Sulphuric acid* which complies with the following additional test :

Dilute 10 g with 50 ml of water, add 0.2 ml of *stannous chloride solution AsT,* and apply the General Test; no visible stain is produced.

Zinc AsT : *Granulated zinc* which complies with following additional test :

Add 10 ml of *stannated hydrochloric acid AsT* to 50 ml of *water,* and apply the General Test, using 10 of the zinc and allowing the action to continue for one hour; no visible stain is produced (limit of arsenic). Repeat the test with the addition of 0.1 ml of *dilute arsenic solution AsT;* a faint but distinct yellow stain is produced (test for sensitivity).

General Method of Testing – By a variable method of procedure suitable to the particular needs of each substance, a solution is prepared from the substance being examined which may or may not contain that substance, but contains the whole of the arsenic (if any) originally present in that substance. This solution, referred to as the `test solution', is used in the actual test.

General Test – The glass tube is lightly packed with cotton wool, previously moistened with *lead acetate solution* and dried, so that the upper surface of the cotton wool is not less than 25 mm below the top of the tube. The upper end of the tube is then inserted into the narrow end of one of the pair of rubber bungs, either to a depth of about 10 mm when the tube has a rounded-off end, or so that the ground end of the tube is flush with the larger end of the bung. A piece of *mercuric chloride paper* is placed flat on the top of the bung and the other bung placed over it and secured by means of the rubber band or spring clip in such a manner that the borings of the two bungs (or the upper bung and the glass tube) meet to form a true tube 6.5 mm in diameter interrupted by a diaphragm of *mercuric chloride paper.*

Instead of this method of attaching the *mercuric chloride paper,* any other method may be used provided (1) that the whole of the evolved gas passes through the paper; (2) that the portion of the paper in contact with the gas is a circle 6.5 mm in diameter; and (3) that the paper is protected from sunlight during the test. The test solution prepared as specified, is placed in the wide-mouthed bottle, 1 g of *potassium iodide AsT* and 10 g of *zinc AsT* added, and the prepared glass tube is placed quickly in position. The action is allowed to proceed for 40 minutes. The yellow stain which is produced on the *mercuric chloride paper* if arsenic is present is compared by day light with the *standard stains* produced by operating in a similar manner with known quantities of *dilute arsenic solution AsT* . The comparison of the stains is made

immediately at the completion of the test. The *standard stains* used for comparison are freshly prepared; they fade on keeping.

By matching the depth of colour with *standard stains*, the proportion of arsenic in the substance may be determined. A stain equivalent to the 1-ml *standard stain*, produced by operating on 10 g of substance indicates that the proportion of arsenic is 1 part per million.

NOTE – (1) The action may be accelerated by placing the apparatus on a warm surface, care being taken that the mercuric chloride paper remains dry throughout the test.

(2) The most suitable temperature for carrying out the test is generally about 40º but because the rate of the evoluion of the gas varies somewhat with different batches zinc AsT, the temperature may be adjusted to obtain a regular, but not violent, evolution of gas.

(3) The tube must be washed with *hydrochloric acid AsT*, rinsed with water and dried between successive tests.

Standard Stains – Solutions are prepared by adding to 50 ml of water, 10 ml of *stannated hydrochloric acid AsT* and quantities of *dilute arsenic solutions AsT* varying from 0.2 ml to 1 ml. The resulting solutions, when treated as described in the General Test, yield stains on the *mercuric chloride paper* referred to as the standard stains.

Preparation of the Test Solution

In the various methods of preparing the test solution given below, the quantities are so arranged unless otherwise stated, that when the stain produced from the solution to be examined is not deeper than the 1-ml *standard stain,* the proportion of arsenic present does not exceed the permitted limit.

Ammonium chloride – Dissolve 2.5 g in 50 ml of *water,* and 10 ml of *stannated hydrochloric acid AsT.*

Boric acid – Dissolve 10 g with 2 g of *citric acid AsT* in 50 ml water, and add 12 ml of *stannated hydrochloric acid AsT.*

Ferrous sulphate – Dissolve 5 g in 10 ml of *water and 15 ml of stannated hydrochloric* acid *AsT and* disitil 20 ml; to the distillate add a few drops of *bromine solution AsT.* Add 2 ml of *stannated hydrochloric acid AsT,* heat under a reflux condenser for one hour, cool, and add 10 ml of *water* and 10 ml of *hydrochloric acid AsT.*

Glycerin – Dissolve 5 g in 50 ml of water, and add 10 ml of *stannated hydrochloric acid AsT.*

Hydrochloric acid – Mix 10 g with 40 ml of water and 1 ml of *stannous chloride solution AsT.*

Magnesium sulphate – Dissolve 5 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT.*

Phosphoric acid – Dissolve 5 g in 50 ml of *water* and add 10 ml of *stannated hydrochloric acid AsT*

Potassium iodide – Dissolve 5 g in 50 ml of *water* and add 2 ml of *stannated hydrochloric acid AsT.*

Sodium bicarbonate – Dissolve 5 g in 50 ml of *water* and add 15 ml of *brominated hydrochloric acid AsT,* and remove the excess of bromine with a few drops of *stannous chloride solution AsT.*

Sodium hydroxide – Dissolve 2.5 g in 50 ml of *water,* add 16 ml of *brominated hydrochloric acid AsT,* and remove the excess of *bromine* with a few drops of *stannous chloride solution AsT.*

2.3.2 –Limit Test for Chlorides

Dissolve the specified quantity of the substance in *water* or prepare a solution as directed in the text and transfer to a *Nessler cylinder.* Add 10 ml of *dilute nitric acid,* except when nitric acid is used in the preparation of the solution, dilute to 50 ml with water, and add 1 ml of *silver nitrate solution.* Stir immediately with a glass rod and allow to stand for 5 minutes. The opalescence produced is not greater than the *standard opalescence,* when viewed transversely.

Standard Opalescence

Place 1.0 ml of a 0.05845 percent w/v solution of *sodium chloride* and 10 ml of *dilute nitric acid* in a *Nessler cylinder*. Dilute to 50 ml with water and add 1 ml of *silver nitrate solution*. Stir immediately with a glass rod and allow to stand for five minutes.

2.3.3 –Limit Test For Heavy Metals

The test for heavy metals is designed to determine the content of metallic impurities that are coloured by sulphide ion, under specified conditions. The limit for heavy metals is indicated in the individual monographs in terms of the parts of lead per million parts of the substance (by weight), as determined by visual comparison of the colour produced by the substance with that of a control prepared from a standard lead solution.

Determine the amount of heavy metals by one of the following methods and as directed in the individual monographs. Method A is used for substances that yield clear colourless solutions under the specified test conditions. Method B is used for substances that do not yield clear, colourless solutions under the test conditions specified for method A, or for substances which, by virtue of their complex nature, interfere with the precipitation of metals by sulphide ion. Method C is used for substances that yield clear, colourless solutions with *sodium hydroxide solutions.*

Special Reagents –

Acetic acid Sp. – *Acetic acid* which complies with the following additional test : Make 25 ml alkaline with *dilute ammonia solution Sp.,* add 1 ml of *potassium cyanide solution Sp.,* dilute to 50 ml with *water* and add two drops of *sodium sulphide* solution; no darkening is produced.

Dilute acetic acid Sp. – *Dilute acetic acid* which complies with the following additional test – Evaporate 20 ml in a porcelain dish, nearly to dryness on a water-bath. Add to the residue 2 ml of the acid and dilute with water to 25 ml, add 10 ml of *hydrogen sulphide solution.* Any dark colour produced is not more than that of a control solution consisting of 2 ml of the acid and 4.0 ml of *standard lead solution* diluted to 25 ml with *water.*

Ammonia solution Sp. – *Strong ammonia solution* which complies with the following additional test : Evaporate 10 ml to dryness on a water-bath; to the residue add 1 ml of *dilute hydrochloric acid Sp. a*nd evaporate to dryness. Dissolve the residue in 2 ml of dilute acetic acid Sp. Add sufficient water to produce 25 ml.

Add 10 ml of *hydrogen sulphide solution.* Any darkening produced is not greater than in a blank solution containing 2 ml of dilute acetic acid Sp. 1.0 ml of *standard lead solution* and sufficient *water* to produce 25 ml.

Dilute ammonia solution Sp. – *Dilute ammonia solution* which complies with the following additional test : To 20 ml add 1 ml of *potassium cyanide solution Sp.,* dilute to 50 ml with *water,* and add two drops of *sodium sulphide solution;* no darkening is produced.

Hydrochloric acid – *Hydrochloric acid* which complies with the following additional test : Evaporate off the acid in a beaker to dryness on a water-bath. Dissolve the residue in 2 ml of *dilute acid Sp.,* dilute to 17

ml with water and add 10 ml of *hydrogen sulphide solution;* any darkening produced is not greater than in a blank solution containing 2.0 ml of *standard lead solution,* 2 ml of *dilute acetic acid Sp.* and dilute to 40 ml with water.

Dilute hydrochloric acid Sp. – *Dilute hydrochloric acid,* which complies with the following additonal test: Treat 10 ml of the acid in the manner described under *Hydrochloric acid Sp.*

Lead nitrate stock solution – Dissolve 0.1598 g of *lead nitrate* in 100 ml of *water* to which has been added 1 ml of *nitric acid,* then dilute with *water* to 1000 ml.

This solution must be prepared and stored in polyethylene or glass containers free from soluble lead salts.

Standard lead solution – On the day of use, dilute 10.0 ml of *lead nitrate stock solution* with *water* to 100.0 ml. Each ml of *standard lead solution* contains the equivalent of 10 µg of lead. A control comparison solution prepared with 2.0 ml of standard lead solution contains, when compared to a solution representing 1.0 g of the substance being tested, the equivalent of 20 parts per million of lead.

Nitric acid Sp. –*Nitric acid* which complies with the following additional test : Dilute 10 ml with 10 ml of *water,* make alkaline with *ammonia solution Sp.,* add 1 ml of *potassium cyanide solution Sp.,* dilute to 50 ml with water, and add two drops of *sodium sulphide solution;* no darkening is produced.

Potassium cyanide solution Sp. – See Appendix 2.3.5.

Sulphuric acid Sp. – Sulphuric acid which complies with following additional test : Add 5 g to 20 ml of *water* make alkaline with *ammonia solution Sp.,* add 1 ml of *potassium cyanide solution Sp.*, dilute to 50 ml with *water* and add two drops of *sodium sulphide solution;* no darkening is produced.

Method A

Standard solution – Into a 50 ml *Nessler cylinder,* pipette 2 ml of *standard lead solution* and dilute with *water* to 25 ml. Adjust with *dilute acetic acid Sp.* or *dilute ammonia solution Sp* to a pH between 3.0 and 4.0, dilute with *water* to about 35 ml, and mix.

Test solution – Into a 50 ml *Nessler cylinder,* place 25 ml of the solution prepared for the test as directed in the individual monogrpah, or using the stated volume of acid when specified in the individual monograph, dissolve and dilute with *water* to 25 ml the specified quantity of the substance being tested. Adjust with *dilute acetic acid Sp.* or *dilute ammonia solution Sp.* to a pH between 3.0 and 4.0, dilute with *water* to about 35 ml and mix.

Procedure – To each of the cylinders containing the *standard solution* and t*est* solution respectively add 10 ml of freshly prepared *hydrogen sulphide solution,* mix, dilute with *water* to 50 ml, allow to stand for five minutes, and view downwards over a white surfac; the colour produced in the *test solution* is not darker than that produced in the *standard solution.*

Method B

Standard solution – Proceed as directed under Method A.

Test solution – Weigh in a suitable crucible the quantity of the substance specified in individual monograph, add sufficient *sulphuric acid Sp.* to wet the sample, and ignite carefully at a low temperature until thoroughly charred. Add to the charred mass 2 ml of *nitric acid Sp.* and five drops of *sulphuric acid Sp.* and heat cautiously until white fumes are no longer evolved. Ignite, preferably in a muffle furnace, at 500º to 600º until the carbon is completely burnt off . Cool, add 4 ml of *hydrochloric acid Sp.,* cover, digest on a water bath for 15 minutes, uncover and slowly evaporate to dryness on a water-bath. Moisten the residue with one drop of *hydrochloric acid Sp.,* add 10 ml of hot water and digest for two minutes. Add

ammonia solution Sp., dropwise, until the solution is just alkaline to *litmus paper,* dilute with *water* to 25 ml and adjust with dilute acetic acid Sp. to a pH between 3.0 and 4.0. Filter if necessary, rinse the crucible and the filter with 10 ml of water, combine the filtrate and washings in a 50 ml *Nessler cylinder,* dilute with *water,* to about 35 ml, and mix. Procedure : Proceed as directed under Method A.

Method C

Standard solution – Into a 50 ml *Nessler cylinder,* pipette 2 ml of *standard lead solution,* add 5 ml of *dilute sodium hydroxide solution.,* dilute with *water* to 50 ml and mix.

Test solution – Into a 50 ml *Nessler cylinder,* place 25 ml of the solution prepared for the test as directed in the individual monograph; or, if not specified otherwise in the individual monograph, dissolve the specified quantity in a mixture of 20 ml of *water* and 5 ml of *dilute sodium hydroxide solution.* Dilute 50 ml with water and mix.

Procedure –To each of the cylinders containing the *standard solution* and the *test solution,* respectively add 5 drops of *sodium sulphide solution,* mix, allow to stand for five minutes and view downwards over a white surface; the colour produced in the *test solution* is not darker than that produced in the *standard solution.*

2.3.4. Limit Test For Iron

Standard iron solution – Weigh accurately 0.1726 g of *ferric ammonium sulphate* and dissolve in 10 ml of 0.1 *N sulphuric acid* and sufficient *water* to produce 1000.0 ml. Each ml of this solution contains 0.02 mg of Fe.

Method

Dissolve the specified quantity of the substance being examined in 40 ml of *water,* or use 10 ml of the solution precribed in the monograph, and transfer to a *Nessler cylinder.* Add 2 ml of a 20 per cent w/v solution of *iron-free citric acid* and 0.1 ml of *thioglycollic acid,* mix, make alkaline with *iron-free ammonia solution,* dilute to 50 ml with *water* and allow to stand for five minutes. Any colour produced is not more intense than the standard colour.

Standard colour – Dilute 2.0 ml of *standard iron solution* with 40 ml of *water* in a *Nessler cylinder.* Add 2 ml of a 20 per cent w/v solution of *iron-free citric acid* and 0.1 ml of *thioglycollic acid,* mix, make alkaline with *iron-free ammonia solution,* dilute to 50 ml with *water* and allow to stand for five minutes.

2.3.5. Limit Test for Lead

The following method is based on the extraction of lead by solutions of dithizone. All reagents used for the test should have as low a content of lead as practicable. All reagent solutions should be stored in containers of borosilicate glass. Glassware should be rinsed thoroughly with warm *dilute nitric acid,* followed by *water.*

Special Reagents

(1) Ammonia-cyanide solution Sp. – Dissolve 2 g of *potassium cyanide* in 15 ml of *strong ammonia solution* and dilute with *water* to 100 ml.

(2) Ammonium citrate solution Sp. – Dissolve 40 g of *citric acid* in 90 ml *water.* Add two drops of *phenol red solution* then add slowly *strong ammonia solution* until the solution acquires a reddish colour. Remove any lead present by extracting the solution with 20 ml quantities of *dithizone extraction solution* until the dithizone solution retains its orange-green colour.

(3) Dilute standard lead solution – Dilute 10.0 ml of *standard lead solution* with sufficient 1 per cent v/v solution of nitric acid to produce 100.0 ml. Each ml of this solution contains 1 µg of lead per ml.

(4) Dithizone extraction solution –Dissolve 30 mg of *diphenylthiocarbazone* in 1000 ml of *chloroform* and add 5 ml of *alcohol.* Store the solution in a refrigerator. Before use, shake a suitable volume of the solution with about half its volume of 1 per cent v/v solution of *nitric acid* and discard the acid.

(5) Hydroxylamine hydrochloride solution Sp. – Dissolve 20 g of *hydroxylamine hydrochloride* in sufficient *water* to produce about 65 ml. Transfer to separator, add five drops of *thymol blue solution*, add *strong ammonia solution* until the solution becomes yellow. Add 10 ml of a 4 per cent w/v solution of *sodium diethyldithiocarbamate* and allow to stand for five minutes. Extract with successive quantities, each of 10 ml, of *chloroform* until a 5 ml portion of the extract does not assume a yellow colour when shaken with dilute copper sulphate solution. Add *dilute hydrochloric acid* until the solution is pink and then dilute with sufficient water to produce 100 ml.

(6) Potassium cyanide solution Sp. – Dissolve 50 g of potassium cyanide in sufficient *water* to produce 100 ml. Remove the lead from this solution by extraction with successive quantities, each of 20 ml of *dithizone extraction solution* until the dithizone solution retains its orange-green colour. Extract any dithizone remaining in the cyanide solution by shaking with *chloroform.* Dilute this cyanide solution with sufficient *water* to produce a solution containing 10 g of *potassium cyanide* in each 100 ml.

(7) Standard dithizone solution – Dissolve 10 ml of *diphenylthiocarbazone* in 1000 ml of *chloroform.* Store the solution in a glass-stoppered, lead-free bottle, protected from light and in a refrigerator.

(8) Citrate-cyanide wash solution – To 50 ml of *water* add 50 ml of *ammonium citrate solution Sp.* and 4 ml of *potassium cyanide solution Sp.,* mix, and adjust the pH, if necessary, with strong *ammonia solution* to 9.0.

(9) Buffer solution pH 2.5 – To 25.0 ml of 0.2 *M potassium hydrogen phthalate add* 37.0 ml of 0.1 N *hydrochloric acid,* and dilute with sufficient *water* to produce 100.0 ml.

(10) Dithizone-carbon tetrachloride solution –Dissolve 10 mg of *diphenylthiocarbazone* in 1000 ml of carbon tetrachloride. Prepare this solution fresh for each determination.

(11) pH 2.5 wash solution – To 500 ml of a 1 per cent v/v *nitric acid* add *strong ammonia solution* until the pH of the mixture is 2.5, then add 10 ml of *buffer solution* pH 2.5 and mix.

(12) Ammonia-cyanide wash solution – To 35 ml of pH 2.5 *wash solution* add 4 ml of *ammonia-cyanide solution Sp.,* and mix.

Method

Transfer the volume of the prepared sample directed in the monograph to a separator, and unless otherwise directed in monograph, add 6 ml of *ammonium citrate solution Sp.,* and 2 ml *hydroxylamine hydrochloride solution Sp.,* (For the determination of lead in iron salts use 10 ml of *ammonium citrate solution Sp.).* Add two drops of *phenol red solution* and make the solution just alkaline (red in colour) by the addition *of strong ammonnia solution.* Cool the solution if necessary, and add 2 ml of *potassium cyanide solution Sp.* Immediately extract the solution with several quantities each of 5 ml, of *dithizone extraction solution,* draining off each extract into another separating funnel, until the dithizone extraction solution retains its green colour. Shake the combine dithizone solutions for 30 seconds with 30 ml of a 1 per cent w/v solution of *nitric acid* and discrad the chloroform layer. Add to the solution exactly 5 ml of *standard dithizone solution* and 4 ml of *ammonia-cyanide solution Sp.* and shake for 30 seconds; the colour of the chloroform layer is of no deeper shade of violet than that of a control made with a volume of *dilute standard lead solution* equivalent to the amount of lead permitted in the sample under examination.

2.3.6 Sulphated Ash

Heat a silica or platinum crucible to redness for 10 minutes, allow to cool in a desiccator and weigh. Put 1 to 2 g of the substance, accurately weighed, into the crucible, ignite gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1 ml of *sulphuric acid,* heat gently until white fumes are no longer evolved and ignite at 800º ± 25º until all black particles have disappeared. Conduct the ignition in a place protected from air currents. Allow the crucible to cool, add a few drops of *sulphuric acid* and heat. Ignite as before, allow to cool and weigh. Repeat the operation until two successive weighings do not differ by more than 0.5 mg.

2.3.7 –Limit Test for Sulphates

Reagents –

Barium sulphate reagent – Mix 15 ml of 0.5 *M barium chloride,* 55 ml of *water,* and 20 ml of *sulphate free alcohol,* add 5 ml of a 0.0181 per cent w/v solution of potassium sulphate, dilute to 100 ml with *water,* and mix. Barium sulphate reagent must be freshly prepared.

0.5 M Barium chloride – *Barium chloride d*issolved in *water* to contain in 1000 ml 122.1 g of $BaCl_2$, $2H_2O$.

Method

Dissolve the specified quantity of the substance in *water* , or prepare a solution as directed in the text, transfer to a *Nessler cylinder,* and add 2 ml of *dilute hydrochloric acid,* except where *hydrochloric acid* is used in the preparation of the solution. Dilute to 45 ml with *water*, add 5 ml of barium sulphate reagent. Stir immediately with a glass rod, and allow to stand for five minutes. The turbidity produced is not greater than the *standard turbidity*, when viewed transversely. Standard turbidity : Place 1.0 ml of 0.1089 per cent w/v solution of potassium sulphate and 2 ml of *dilute hydrochloric acid* in a *Nessler cylinder,*dilute to 45 ml with *water*, add 5 ml of barium sulphate reagent, stir immediately with a glass rod and allow to stand for five minutes.

APPENDIX –3

3.1 PHYSICAL TESTS AND DETERMINATIONS

3.1 Determination of Boiling or Distilling Range

The boiling range of a liquid is the temperature interval, corrected for a pressure of 760 torr within which the liquid or a specified fraction of the liquid, distils under the conditions specified in the test. The lower limit of the range is the temperature indicated by the thermometer when the first drop of condensate leaves the tip of the condenser, and the upper limit is the temperature at which the last drop evaporates from the lowest point in the distillation flask without taking into account any liquid remaining On the side. of the flask; it may also be the temperature observed when the proportion specified in the individual has been collected.

Apparatus-

Use an apparatus consisting of the following

I. Distilling flask : A round-bottom distilling flask of 200 ml capcity and having a total length of 17 to 19 em and an inside neck diameter of 20 to 22 mm. Attached about midway on the neck approximately 12 em from the bottom of the flask, is a side-arm 10 to 12 em long and 5 mm in internal diameter which is at an angle of 70° to 75° with the lower portion of the neck.

2. Condenser : A straight glass condenser 55 to 60 em long with a water-j : cket about 40 em long Or any other type of condensor having equivalent condensing capacity. The lower end of the condenser may be bent to provide a delivery tube, or it may be connected to a bent adaptor that serves as a delivery tube.

3.Receiver : A 100 ml cylinder, graduated in I ml sub-divisions.

4.Thermometer: An accurately standardised, partial immersion thermometer having the smallest practical sub-divisions (not greater than 0.2°). When placed in position, the steam is located in the centre of the neck and the top of the bulb is just below the bottom of the outlet to the side arm.

Method

If the liquid under examination distils below 80°, cool it to between 10° and ISO before measuring the sample for distillation.

Assemble the apparatus, and place in the flask 100 ml of the liquid under examination, taking care not to allow any of the liquid to enter the side-arm. Insert the thermometer and seal the entire heating and flask assembly from external air currents. Add a few pieces of porous material and heat rapidly to boiling using a Bunsen burner and an asbestos plate pierced by a hole 33 mm in diameter. Record the temperature at which the first drop of distillate falls into the cylinder, and adjcst the rate of heating to obtain a regular distillation rate of 4 to 5 ml per minute. Record the temperature when the last drop of liquid evaporates from the bottom of the flask or when the specified percentage has distilled over. Correct the observed temperature readings for any variation in the barometric pressure from the normal (760 torr) using the following expression :

$$t.=t_2+k(a-b)$$

where

t_4 = the corrected temperature

t_2 = the observed temperature

a = 760 (torr)

b= the Barometric pressure in torr at the time of determination

K= the correction factor indicated in the following table :

Distillation range	k
Less than 100°	0.04
100° to 140°	0.045
140° to 190°	0.5
190' to 240°	0.055
More than 240°	0.06

3.2 Determmation of congealing range or temperature

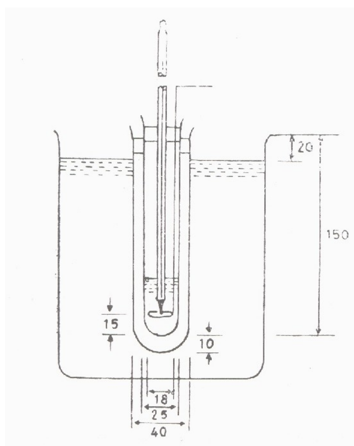
The congealing temperature is that point at which there exists a mixture of the liquid (fused) phase of a substance and a small but increasing proportion of the solid phase. It is distinct from the freezing point, which is the temperature at which the liquid and solid phase of a substance are in equilibrium.

The temperature at which a substance solidifies upon cooling is a useful index of its purity if heat is liberated when solidification takes place.

The following method is applicable to substances that melt between -20° and 150°

Apparatns-

A test-tube about 25 ;mm in diameter and 150 mm long placed inside a test tube about 40 mm in diameter and 160 mm long; the inner tube is closed by a stopper that carries a stirrer and a thermometer



(Dimensions in mm)

(about 175 mm long and with 0.2 graduations) fixed, so that the bulb is about 15 mm above the bottom of the tube. The stirrer is made from a glass rod or other suitable material formed at one end into a loop of about 18 mm overall diameter at right angle to the rod. The inner tube with its jacket is supported centrally in a l-litre beaker containing a suitable cooling liquid to within 20 mm of the top. A thermometer is supported in the cooling bath (see Fig. 5).

Melt the substance, if solid, at a temperature not more than 20· above its expected congealing point and pour it into the inner test-tube to a height of 50 to 57 mm. Assemble the apparatus with the bulb of the thermometer immersed half-way between the top and bottom of the sample in the test-tube. Fill the bath to almost 20 mm from the tube with a suitable fluid at a temperature 4° to 50° below the expected congealing point. If the substance is a liquid at room temperature, carry out the determination using a bath temperature about 15 below the expected congealing point. When the sample has cooled to about 5° above its expected congealing point stir it continuously by moving the loop up and down between the top and bottom of the sample, at a regular rate of 20 complete cycles per minute. Record the reading of the thermometer every 30 seconds and continue stirring only 110 long as the temperature is falling. Stop the stirring when the temperature is constant or starts to rise slightly. Continue recording the temperature for at least three minutes after the temperature again begins to fall after remaining constant.

The congealing point willlbe the average of not less than four consecutive readings that lie: within range of 0.2°.

3.3 Determination of pH Values

The pH value conventionally represents the acidity or alkalinity of an aqueous solution. In the pharmacopoeia, standards and limits on pH have been provided for these pharmacopoeial substances in which pH as a measure of the hydrogen activity is important from the stand point of stability or physiological suitability.

The measurement of pH is generally done with a suitable potentiometric meter known as the pH meter fitted with two electrodes, one constructed of glass and sensitive to hydrogenation activity and the other a calomel reference electrode. The determination i. carried out at temperature of 254° ± 2°, un less otherwise specified in the individual monograph.

Apparatus-

The pH value of a solution is determined potentiometrically by means of a glass electrode, a reference electrode and a pH meter either of the potentiometric or of the deflection type.

Operate the pH meter and electrode system according to the manufacturer's instructions. Calibrate the apparatus using buffer *solution D* as the primary standard, adjusting the meter to read the appropriate pH value given in the Table I, corresponding to the temperature of the solution. Where provision is made for setting the scale, use a second reference buffer solution, either *buffer solution A, buffer solution E* or *buffer solution* G. In this case a check is carried out with a third referene buffer solution of intermediate pH, when the reading of the intermediate solution must not differ by more than 0.05 pH unit from the corresponding value indicated in the Table. Where there is no provision for setting the scale with a second reference buffer solution, checks should be made with two reference buffer solutions, the readings for which must not differ by more than 0.05 pH unit from the value corresponding to each solution.

TABLE 1 - pH of Reference Solutions at various Temperatures

Temperature	Buffer Solutions							
t °	A	B	C	D	E	F	G	H
15	1.67	-	3.80	4.00	6.90	7.45	9.28	10.12
20	1.68	-	3.79	*4.00*	6.38	7.43	9.22	10.03
25	1. 68	3.56	3.78	4.01	6.86	7.41	9.18	10.01
30	1.68	3.55	3.77	4.02	6·85	7.40	9.14	9.97
35	1.69	3.55	3.76	4.02	6.84	7.39	9.10	9.98
ΔpH/Δt	+0.001	-0.001	-0.002	+ 0.001	-0.003	+0.003	-0.008	-0.009

Reference buffer solutions

The following reference buffer solutions must be prepared using *carbon dioxode free water;* phthalate and phosphate salts should be dried at 110° for two hours before use. Buffer solutions should be stored in bottles made of alkali-free glass, and must not be used later than three months after preparation.

1.Buffer solution A : Dissolve 12.71 g of *potassium tetraoxalate* in sufficient *carbon dioxide-free water* to produce 1000.0 ml.

2.Buffer solution B : A freshly prepared saturated solution, at 25°, of *potassium hydrogen tartrate.*

3.Buffer solution C : Dissolve 11.51 g of *potassium dihydrogen citrate* in sufficient carbon dioxide free water to produce 1000.0 ml.

Note - *This solution must be freshly prepared.*

4. Buffer solution D : Dissolve 10.21 g of *potassium hydrogen phthalate* in sufficient *carbon dioxidefree water* to produce 1000.0 ml.

5. Buffer solution E : Dissolve 3.40 g of *potassium dihydrogen phosphate* and 3.55 g of *anhydrous disodiurn hydrogen phosphate,* both previously dried at 110° to 130° for two hours, in sufficient

carbon dioxide-free water to produce 1000.0 ml.

6. Buffer solution F: Dissolve 1.184 g of *potassium dihydrogen phosphate* and 4.303 g *of anhydrous disodium hydrogen phosphate,* both previously dried at 110° to 130° for two hours, in sufficient *carbon dioxide-free water* to produce 1000.0 ml.

7. Buffer solution G : Dissolve 3.814 g of *borax* in sufficient *carbon dioxide-free water* to produce 1000.0 ml.

Note-This solution should be stored protected from carbon dioxide.

8. Buffer solution H : Dissolve 7. 155 g of *sodium carbonate* and 2. 10 g of *sodium bicarbonate* in sufficient *carbon dioxide-free water* to produce 1000.0 ml.

Method

Immerse the electrodes in the solution to be examined and measure the pH at the same temperature as for the standard solutions. At the end of a set of measurements, take a reading of the solution used to standardise the meter and electrodes. If the difference between this reading and the original value is greater than 0.05, the set of measurements must be repeated.

When measuring pH values above 10 .0, ensure that the glass electrode is suitable for use under alkaline conditions and apply any correction that is necessary.

All solutions of substances being examined must be prepared using *carbon dioxide-free water* .

3.4 Determination of melting range or temperature

In this Pharmacopoeia, melting range or temperature of a substance is defined as those points of ternperature within which, or the point at which, the substance begins to coalesce and is completely melted except as defined otherwise for certain substance. The following procedures are suitable for the various substances described in the Pharmacopoiea. Any other apparatus or method capable of the same accuracy may also be used. The accuracy should be checked frequently by the use of One of the following reference substances, that melts nearest to the melting range of thc substance to be tested:

	Melting range
Vanillin	81°-83°
Acetanilide	141°-116°
Phenacetin	134°-136°
Sulphanilarnide	164.5°-166.5°
Sulphapyridine	191°-193°
Caffeine (dried at 100°)	234°-237°

Unless otherwise specified in the individual monograph, Method I should be used.

Method I Apparatus

(a) A glass heating vessel of suitable construction and capacity containing one of the following or any other suitable bath liquid, to a height of not less than 14 cm.

(i) Water for temperatures upto 60°

(ii) Glycerin for temperatures upto 150°.

(iii) Liquid paraffin for sufficiently high boiling range for temperatures upto 250°. (iv) Sesame oil or a suitable grade of liquid silicone for temperatures upto 300°.

(b) A suitable stirring device, capable of rapidly mixing the liquids.

(c) An accurately standardised thermometer suitable for the substance. under examination :(see Appendix 1.3). The thermometer must be positioned In the bath liquid to Its specified immersion depth and yet leave the bulb at about 2 cm above the bottom of the bath.

(d) Thin-walled capillary glass tubes of hard glass, about 12 cm long, with a well thickness of 0.2 to 0.3mm and an internal diameter of 0.8 to 1.1 mm. The tubes should preferably be kept sealed at both ends and cut as required.

(e) Source of heat (open flame or electric heater).

Procedure : Reduce the substance to a very fine powder and unless otherwise directed, dry it at a temperature considerably below its melting temperature or under reduced pressure Over a suitable desiccant for not less than 16 hours. Introduce into a capillary glass tube, one end of which is sealed, a sufficient quantity of the dry powder to form a compact column about 3 mm high.

Heat the bath until the temperature is about 10' below the expected melting point. Remove the thermometer and quickly attach the capillary tube to the thermometer by wetting both with a drop of the liquid of the bath or otherwise and adjust its height so that the closed end of the capillary is near the middle of the thermometer bulb. Replace the thermometer and continue the heating, with constant stirring, sufficiently to cause the temperature to rise at a rate of about 3' per minute. When the temperature is about 3' below the lower limit of the expected melting range, reduce the heating so that the temperature rises at the rate of about 1° to 2° per minute. Continue the heating and note the temperature at which the column of the sample collapses definitely against the side of the tube at any point, when melting may be considered to have begun and note also the temperature at which the sample becomes liquid throughout as seen by the formation of a definite meniscus. The two temperatures fall within the limits of the melting range.

Method II

Apparatus : Use the apparatus described under Method I except that the glass capillary tube is open at both ends and has an internal diameter of 1.1 to 1.3 mm, an external diameter of 1.4 to 1.3 mm and length of 50 to 60 mm.

Procedure : Rapidly melt the material to be tested, at a temperature not more than 10° above the point of complete fusion. Draw it into a capillary tube to a depth of about 10 mm. Cool the charged tube at 10°, or lower, for 24 hours, or in contact with ice for at least 2 hours. Attach the tube to the thermometer and adjust it so that the column of substance is in level with the thermometer bulb; suspend the thermometer in the heating vessel containing water at 15° so that the lower end of the column of the substance is 30 mm below the surface of the water and heat the water with constant stirring so that the temperature rises at the rate of l° per minute the temperature at which the partly melted substance is observed to rise in the capillery tube is the melting temperature.

Method III

Apparatus: (a) A glass boiling-tube, overall length, l10 mm , internal diameter, 25 mm.

(b) A cork about 25 mm long to fit into the boiling-tube, bored with a central bole to fit the standard thermometer and with a grove cut in the side.

(c) A glass beaker, of such a size that when the apparatus is assembled, the boiling-tube can be immersed vertically to two-thirds of its length in the water in the beaker with its lower end about 2.5 cm above the bottom of the beaker.

(d) A stirrer or any other device which will ensure uniformity of the temperature throughout the water in the beaker.

(e) An accurately standardised thermometer suitable for the substance under examination (see Appendix 1.3).

(f) Suitable means of heating the water in the beaker.

Procedure: Melt a quantity of the substance slowly, while stirring, until it reaches a temperature of about 90'. Cool and allow the temperature of the molten substance to drop to a temperature of 8' to 10' above the expected melting point. Chill the bulb of the thermometer to 5', wipe it dry and while it is still cold, dip it in the molten substance so that the lower half of the bulb is submerged. Withdraw it immediately, and hold it vertically away from the beat until the wax surface dulls, then dip it for five minutes into a water-bath at a temperatnre not higher than 15°C

Fit the thermometer through the boared cork into the boiling tube so that the lower part is 15 mm above the bottom of the tube. Suspend the tube in the beaker filled with water adjusted to about 15v and raise the temperature of the bath at the rate of 2° per minute to 30°, then adjust the rate to 1 per minute and note the temperatures at which the first drop of melted substance leaves the thermometer. Repeat the determination twice On a freshly melted portion of the substance. If the three readings differ by less than 1', take the average of the three as the melting point. If they differ by more than I', make two additional determinations and take the average of the five readings.

3.5 Optical rotation and specific optical rotation

Optical rotation 'α' is the property shown by certain substances of rotating the plane of polarisation of polarised light. Such substances are said to be optically active in the sense that they cause incident polarised light to emerge in a plane forming a measureable angle with the plane of the incident light. Where this effect is large enough for measurement, it may serve as the basis for identifying or assaying a substance.

The *optical rotation* of a substance is the angle through which the plane of polarisation is rotated when polarised light passes through the substance, if liquid, or a solution of the substance. Substances are descri bed as *dextra-rotatory* Or laevo-rotatory according to whether the plane of polarisation is rotated clockwise or anticlockwise, respectively, as determined by viewing towards the light Source. *Dextra-rotation* is designated (+) and laevo-rotation is designated (-).

The *optical rotation,* unless otherwise specified, is measured at the wavelength of the D line of sodium (λ=589.3μm) at 25°, on a layer ldm thick. It is expressed in degrees.

The *specific optical rotation* $(\alpha)^{25}_{D}$ of a liquid substance is the angle of rotation ex: of the plane of polarisation at the wavelength of the D line of sodium (A-589.3 mm) measured at 25°, calculated with reference to a 1.0 dm thick layer of the liquid, and divided by the specific gravity.

The *specific optical rotation* $(\alpha)^{25}_{D}$ of a solid substance is the angle of rotation *a* of the plane of polarisation at the wavelength of the D line of sodium measured at 25° and calculated with reference to a layer 1.0 dm thick of a solution containing 1 g of the substance per ml, The specific optical rotation of a solid is always expressed with reference to a given solvent.

Apparatus

A commercial instrument constructed for use with a sodium lamp and capable of giving readings to the nearest 0.02° is suitable for most purposes. For certain applications, the use of a photo-electric polarimeter capable of taking measurements at the specified wave length may be necessary.

The accuracy and precision of optical rotation measurements can be increased if the following precautions are taken :

(a) The instrument must be in a good condition. Optical elements must be very clean and in exact alignment. The match point should be close to the normal zero mark.

(b) The light source must be properly aligned with respect to the optical bench. It should be supplemented by a filtering system capable of isolating the D line from sodium light.

(c) Specific attention should be paid to temperature control of the solution and of the polarimeter. (d) Differences between the initial readings Or between observed and corrected optical rotation caculated as either specific optical Or optical rotation should not be more than One fourth of th : range specified in the monograph for the substance.

(e) Polarimeter tubes should be filled in such a way as to avoid air bubbles. Particular care is nece**ssary for semi-micro or micro tubes.**

(f) For tubes with removable end-plates fitted with gaskets and caps, tighten the end-plates only enough to ensure a leak-proof seal between the end-plate and the body of the tube.

(g) For substances with low rotatory power, the end plates should be loosened and tightened again after each reading, in the measurement of both the rotation and the zero point.

(h) Liquids and solutions of solids must be clear.

Calibration : The apparatus may be checked by using a solution of previously dried sucrose and measuring the optical rotation in a 2 dm tube at 25° and using the concentrations indicated below:

Concentration g/100 ml	Angle of rotation (+), at 25°
10.0	13.33
20.0	26.61
30.0	39.86
40.0	53.06
50.0	66.23

Method

For solids: Weigh accurately a suitable quantity of the substance being examined, to give a solution of the strength specified in the monograph, and transfer to a volumetric flask by means of *water* Or other solvent if specified. If a solvent is used, reserve a portion of it for the blank determination. Unless otherwise specified, adjust the contents of the flask to 25° by suspending the flask in a constant-temperature bath. Make up to volume with the solvent at 25° and mix well. Transfer the solution to the polarimeter tube within 30 minutes from the time of the substance was dissolved and during this time interval maintain the solution at 25°.

Determine the zero point of the polarimeter and then make five readings of the observed rotation of the test solution at 25°. Take an equal number of readings in the same tube with the solvent in place of the test solution. The zero correction is the average of the blank readings, and is subtracted from the average observed rotation if the two figures are of the same sign Or added if they are opposite in sign, to give the corrected observed rotation.

For liquids: Unless otherwise specified, adjust the temperature of the substance being examined to 25° transfer to a polarimeter tube and proceed as described. For solids, beginning at the words "Determine
the zero point ....".

Calculation-Calculate the specific optical rotation using the following formula. dextro-rotation and laevo-rotation being designated by (+) and (-) respectively:

For liquids $(\alpha)^{25}_{D} = \alpha / Id^{25}_{25}$

For solids $(\alpha)^{25}_{D} = 100\alpha / Ic$

Where

a = corrected observed rotation, in degrees, at 25°

D = D line of sodium light (λ=589.3 mm)

= length of the polarimeter tube in dm.

$(\alpha)^{25}_{D}$ specific gravity of the liquid or solution at 25°

c = concentration of the substance in per cent *wlv*

NOTE-THE REQUIREMENTS FOR OPTICAL ROTATION AND SPECIFIC OPTICAL ROTATION IN THE PHARMACOPOEIA APPLY TO THE DRIED, ANHYDROUS OR SOLVENT FREE MATERIAL.

3.6 Powder Fineness

The degree of coarseness or fineness of a powder is expressed by reference to the nominal mesh aperture size of the sieves for measuring the size of the powders. For practical reasons, the use of sieves, Appendix 1.2, for measuring powder fineness for most pharmaceutical purposes, is convenient but device other than sieves must be employed for the measurement of particles less than 100 mm in nominal size.

The following terms are used in the description of powders :

Coarse powder – A powder, all the particles of which pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 µm.

Moderately coarse powder – A powder, all the particles of which pass through a sieve with a nominal mesh aperture of 710 µm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 250 µm.

Moderately fine powder – A powder, all the particles of which pass through a sieve with a nominal mesh aperture of 355 µm and not more than 40.0 per cent through a sieve with a nominal mesh aperature of 180 µm.

Fine powder – A powder, all the particles of which pass through a sieve with a nominal mesh aperture of 180 µm.

Very fine powder – A powder, all the particles of which pass through a sieve with a nominal mesh aperture of 125 µm .

When the fineness of a powder is described by means of a number, it is intended that all the particles of the powder shall pass through a *sieve* of which the nominal mesh aperture, in µm, is equal to that number.

When a batch of a vegetable drug is being ground and sifted, no portion of the drug shall be rejected but it is permissible except in the case of assays, to withhold the final tailings, if an approximately equal amount of tailings from a preceding batch of the same drug has been added before grinding.

Sieves – Sieves for testing powder fineness comply with the requirements stated under sieves, Appendix 1.2.

Method

(1) For coarse and moderately coarse powders – Place 25 to 100 g of the powder being examined upon the appropriate sieve having a close fitting receiving pan and cover. Shake the sieve in a rotary horizontal direction and vertically by tapping on a hard surface for not less than twenty minutes or until sifting is practically complete. Weigh accurately the amount remaining on the sieve and in the receiving pan.

(2) For fine and very fine powder – Proceed as described under coarse and moderately coarse powders, except that the test sample should not exceed 25 g and except that the sieve is to be shaken for not less than thirty minutes, or until sifting is practically complete.

NOTE – AVOID PROLONGED SHAKING THAT WOULD RESULT IN INCREASING THE FINENESS OF THE POWDER DURING THE TESTING.

With oily or other powders which tend to clog the openings, carefully brush the screen at interval during siftings. Break up any lumps that may form. A mechanical sieve shaker which reproduces the circular and tapping motion given to sieves in hand sifting but has a uniform mechanical action may be employed.

3.7 Refractive Index

The refractive index (n) of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. It varies with the wavelength of the light used in its measurement.

Unless otherwise prescribed, the refractive index is measured at 25º(±0.5) with reference to the wavelength of the D line of sodium (ψ =589.3 nm). The temperature should be carefully adjusted and maintained since the refractive index varies significantly with temperature.

The Abbe refractometer is convenient for most measurements of refractive index but other refractometer of equal or greater accuracy may be used. Commercial refractometers are normally constructed for use with white light but are calibrated to give the refractive index in terms of the D line of sodium light.

To achieve accuracy, the apparatus should be calibrated against *distilled water :* which has a refractive index of 1.3325 at 25º or against the reference liquids given in the following table :-

TABLE

Reference Liquid	$n^{2\sigma\zeta}_{D}$	Temperature Co-efficient $\Delta n/\Delta t$
Carbon tetrachloride	1.4603	-0.00057
Toluene	1.4969	-0.00056
a-Methylnaphthalene	1.6176	-0.00048

* Reference index value for the D line of sodium, measured at 20º

The cleanliness of the instrument should be checked frequently by determining the refractive index of distilled water which at 25º is 1.3325.

3.7 Weight Per Millilitre and Specific Graveity

Weight per milliliter – The weight per millilitre of a liquid is the weight in g of 1 ml of a liquid when weighed in air at 25º, unless otherwise specified.

Method

Select a thoroughly clean and dry pycnometer. Calibrate the pycnometer by filling it with recently boiled and cooled *Water* at 25º and weighing the contents. Assuming that the weight of 1 ml of *water* at 25º when weighed in air of density 0.0012 g per ml, is 0.99602 g. Calculate the capacity of the pycnometer. (Ordinary deviations in the density of air from the value given do not affect the result of a determination significantly). Adjust the temperature of the substance to be examined, to about 20º and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25º, remove any excess of the substance and weigh. Substract the tare weight of the pycnometer from the filled weight of the pycnometer. Determine the weight per milliliter dividing the weight in air, expressed in g, of the quantity of liquid which fills the pycnometer at the specified temperature, by the capacity expressed in ml, of the pycnometer at the same temperature.

Specific gravity –The specific gravity of a liquid is the weight of a given volume of the liquid at 25º (unless otherwise specified) compared with the weight of an equal volume of water at the same temperature, all weighings being taken in air**.**

Method

Proceed as described under Wt. Per ml. Obtain the specific gravity of the liquid by dividing the weight of the liquid contained in the pycnometer by the weight of water contained, both determined at 25º unless otherwise directed in the individual monograph.

APPENDIX –4

4. REAGENTS AND SOLUTIONS

Acetic Acid – Contains approximately 33 per cent w/v of $C_2H_4O_2$. Dilute 315 ml of glacial acetic acid to 1000 ml with *water*.

Acetic Acid, x N – Solutions of any normality xN may be prepared by diluting 60x ml of glacial acetic acid to 1000 ml with water.

Acetic Acid, Dilute – Contains approximately 6 per cent w/w of $C_2H_4O_2$. Dilute 57 ml of glacial acetic acid to 1000 ml with water.

Acetic Acid, Glacial – CH_3COOH =60.05.

Contains not less than 99.0 per cent w/w of $C_2H_4O_2$. About 17.5 N in strength.

Description – At temperature above its freezing point a clear colourless liquid, odour, pungent and characteristic; crystallises when cooled to about 10º and does not completely re-melt until warmed to about 15º.

Solubility – Miscible with *water,* with *glycerin* and most fixed and volatile oils.

Boiling range –Between 117º and 119º.

Congealing temperature –Not lower than 14.8º.

Wt. per ml –At 25º about 1.047 g.

Heavy metals –Evaporate 5 ml to dryness in a porcelain dish on water-bath, warm the residue with 2 ml of 0.1 *N hydrochloric acid* and water to make 25 ml; the limit of heavy metals is 10 parts per million, Appendix 2.3.3.

Chloride –5 ml complies with the limit test for chlorides, Appendix 2.3.2.

Sulphate –5 ml complies with the limit test for sulphates, Appendix 2.3.7.

Certain aldehydic substances – To 5 ml add 10 ml of *mercuric chloride solution* and make alkaline with *sodium hydroxide solution,* allow to stand for five minutes and acidify with dilute *sulphuric acid*; the solution does not show more than a faint turbidity.

Formic acid and oxidisable impurities – Dilute 5 ml with 10 ml of water, to 5 ml of this solution add 2.0 ml of 0.1 *N* potassium dichromate and 6 ml of sulphuric acid, and allow to stand for one minute, add 25 ml of water, cool to 15º, and add 1 ml of freshly prepared potassium iodide solution and titrate the liberated iodine with 0.1 *N* sodium thiosulphate, using starch solution as indicator. Not less than 1 ml of 0.*N sodium thiosulphate* is required.

Odorous impurities –Neutralise 1.5 ml with *sodium hydroxide solution*; the solution has no odour other than a faint acetous odour.

Readily oxidisable impurities – To 5 ml of the solution prepared for the test for Formic Acid and Oxidisable Impurities, add 20 ml of water and 0.5 ml of 0.1 *N potassium permanganate;* the pink colour does not entirely disappear within half a minute.

Non-volatile matter – Leaves not more than 0.01 per cent w/w of residue when evaporated to dryness and dried to constant weight at 105º.

Assay –Weigh accurately about 1 g into a stoppered flask containing 50 ml of *water* and titrate with *N sodium hydroxide,* using *phenolphthalein solution* as indicator. Each ml of *sodium hydroxide* is equivalent to 0.06005 g of C2H4O2.

Acetic Acid, Lead-Free –Acetic acid which complies with following additional test, boil 25 ml until the volume is reduced to about 15 ml, cool make alkaline with lead-free ammonia solution, add 1 ml of lead free *potassium cyanide solution, dilute* to 50 ml with water, add 2 drops of *sodium sulphide solution;* no darkening is produced.

Acetone – Propan 2-one; $(CH_3)_2CO$ = 58.08

Description – Clear, colourless, mobile and volatile liquid; taste, pungent and sweetish; odour characteristic; flammable.

Solubility –Miscible with *water*, with alcohol, with *solvent ether,* and with *chloroform,* forming clear solutions.

Distillation range – Not less than 96.0 per cent distils between 55.5º and 57º.

Acidity– 10 ml diluted with 10 ml of freshly boiled and cooled water; does not require for neutralisation more than 0.2 ml of 0.1 *N sodium hydroxide*, using phenolphthalein solution as indicator.

Alkalinty – 10 ml diluted with 10 ml of freshly boiled and cooled water, is not alkaline to litmus solution.

Methyl alcohol –Dilute 10 ml with water to 100 ml. To 1 ml of the solution add 1 ml of *water* and 2 ml of *potassium permanganate* and *phosphoric acid solution*. Allow to stand for ten minutes and add 2 ml of *oxalic acid* and *sulphuric acid solution;* to the colourless solution add 5 ml of *decolorised magenta solution* and set aside for thirty minutes between 15º and 30º; no colour is produced.

Oxidisable substances –To 20 ml add 0.1 ml of 0.1 *N potassium permanganate,* and allow to stand for fifteen minutes; the solution is not completely decolorised.

Water – Shake 10 ml with 40 ml of *carbon disulphide*; a clear solution is produced.

Non-volatile matter –When evaporated on a water-bath and dried to constant weight at 105º, leaves not more than 0.01 per cent w/v residue.

Acetone Solution, Standard – A 0.05 per cent v/v solution of acetone in water.

Alcohol –

Description –Clear, colourless, mobile, volatile liquid, odour, characteristic and spirituous; taste, burning, readily volatilised even at low temperature, and boils at about 78º, flammable. Alcohol containing not less than 94.85 per cent v/v and not more than 95.2 per cent v/v of C_2H_5OH at 15.56º.

Solubility –Miscible in all proportions with *water,* with *chloroform* and with *solvent ether.*

Acidity or alkalinity – To 20 ml add five drops of *phenolphthalein solution*; the solution remains colourless and requires not more than 2.0 ml of 0.1*N sodium hydroxide* to produce a pink colour.

Specific gravity –Between 0.8084 and 0.8104 at 25º.

Clarity of solution –Dilute 5 ml to 100 ml with *water* in glass cylinder; the solution remains clear when examined against a black background. Cool to 10º for thirty minutes; the solution remains clear.

Methanol – To one drop add one of water, one drop of *dilute phosphoric acid,* and one drop of *potassium permanganate solution.* Mix, allow to stand for one minute and add sodium bisulphite solution dropwise, until the permanganate colour is discharged. If a brown colour remains, add one drop of *dilute phosphoric* acid. To the colourless solution add 5 ml of freshly prepared *chromotropic acid* solution and heat on a water-bath at 60º for ten minutes; no violet colour is produced.

Foreign organic substances – Clean a glass-stoppered cylinder thoroughly with *hydrochloric acid,* rinse with water and finally rinse with the alcohol under examination. Put 20 ml in the cylinder, cool to about 15º and then add from a carefully cleaned pipette 0.1 ml 0.1 *N potassium permanganate.* Mix at once by inverting the stoppered cylinder and allow to stand at 15º for five minutes; the pink colour does not entirely disappear.

Isopropyl alcohol and t-butyl alcohol – To 1 ml add 2 ml of water and 10 ml of *mercuric sulphate solution* and heat in a boiling water-bath; no precipitate is formed within three minutes.

Aldehydes and ketones – Heat 100 ml of *hydroxylamine hydrochloride solution* in a loosely stoppered flask on a water-bath for thirty minutes, cool, and if necessary, add sufficient 0.05 *N sodium hydroxide* to restore the green colour. To 50 ml of this solution add 25 ml of the alcohol and heat on a water bath for ten minutes in a loosely stoppered flask. Cool, transfer to a Nesseler cylinder, and titrate with 0.05 *N sodium hydroxide* until the colour matches that of the remainder of the *hydroxylamine hydrochloride solution* contained in a similar cylinder, both solutions being viewed down the axis of the cylinder. Not more than 0.9 ml of 0.05 *N sodium hydroxide* is required.

Fusel oil constituents – Mix 10 ml with 5 ml of *water* and 1 ml of *glycerin* and allow the mixture to evaporate spontaneously from clean, odourless absorbent paper; no foreign odour is perceptible at any stage of the evaporation.

Non-volatile matter – Evaporate 40 ml in a tared dish on a water-bath and dry the residue at 105º for one hour; the weight of the residue does not exceed 1 mg.

Storage – Store in tightly-closed containers, away from fire.

Labelling – The label on the container states "Flammable".

Dilute Alcohols : Alcohol diluted with water to produce dilute alcohols. They are prepared as described below :

Alcohol (90 per cent)
Dilute 947 ml of alcohol to 1000 ml with water.
Specific Gravity –At 15.56º/15.56º, 0.832 to 0.835, Appendix 3.8 Alcohol (80 per cent)

Alcohol (80 per cent)
Dilute 842 ml of alcohol to 1000 ml with water.
Specific Gravity –At 15.56º/15.56º, 0.863 to 0.865, Appendix 3.8 Alcohol (60 per cent)

Alcohol (60 per cent)
Dilute 623 ml of alcohol to 1000 ml with water.
Specific Gravity –At 15.56º/15.56º, 0.913 to 0.914, Appendix 3.8 Alcohol (50 per cent)

Alcohol (50 per cent)
Dilute 526 ml of alcohol to 1000 ml with water
Specific Gravity –At 15.56º/15.56º, 0.934 to 0.935. Appendix 3.8 Alcohol (25 per cent)

Alcohol (25 per cent)
Dilute 263 ml of alcohol to 1000 ml with water.
Specific Gravity –At 15.56º/15.56º, 0.9705 to 0.9713. Appendix 3.8 Alcohol 20 per cent)

Alcohol (20 per cent)
Dilute 210 ml of alcohol to 1000 ml with water.
Specific Gravity –At 15.56º/15.56º, 0.975 to 0976. Appendix 3.8

Alcohol, Aldehyde-free. –Alcohol which complies with the following additional test :

Aldehyde – To 25 ml, contained in 300 ml flask, add 75 ml of *dinitrophenyl hydrazine solution,* heat on a water bath under a reflux condenser for twenty four hours, remove the alcohol by distillation, dilute to 200 ml with a 2 per cent v/v solution of sulphuric acid, and set aside for twenty four hours; no crystals are produced.

Alcohol, Sulphate-free. –Shake alcohol with an excess of anion exchange resin for thirty minutes and filter.

Ammonia, xN. –Solutions of any normality xN may be prepared by diluting 75 x ml of strong ammonia solution to 1000 ml with water.

Ammonia-Ammonium Chloride Solution, Strong. –Dissolve 67.5 g of *ammonium chloride* in 710 ml of strong *ammonia solution* and add sufficient *water* to produce 1000 ml.

Ammonia Solution, Dilute. – Contains approximately 10 per cent w/w of NH_3.

Dilute 425 ml of *strong ammonia solution* to 1000 ml with *water*.

Wt. per ml – At 25º, about 0.960 g.

Storage – Dilute ammonia solution should be kept in a well-closed container, in a cool place.

Ammonia Solution 2 per cent –Ammonia solution 2 per cent is the ammonia solution strong diluted with purified water to contain 2 per cent v/v of Ammonia solution strong.

Ammonia Solution, Strong –Contains 25.0 per cent w/w of NH3 (limit, 24.5 to 25.5). About 13.5 N in strength.

Description –Clear, colourless liquid; odour, strongly pungent and characteristic.

Solubility –Miscible with *water* in all proportions.

Wt. per. ml – At 25º, about 0.91g.

Heavy metals –Evaporate 5 ml to dryness on a water-bath. To the residue, add 1 ml of *dilute hydrochloric acid* and evaporate to dryness. Dissolve the residue in 2 ml of *dilute acetic acid* and add *water* to make 25 ml; the limit of heavy metals is 15 parts per million, Appendix 2.3.3.

Iron –Evaporate 40 ml on a water-bath to about 10 ml. The solution complies with the *limit test for iron*, Appendix 2.3.4

Chloride –Evaporate 40 ml on a water-bath to about 5 ml. The solution complies with the *limit test for chlorides*, Appendix 2.3.2.

Sulphate –Evaporate 20 ml on a water-bath to about 5 ml. The solution complies with *the limit test for sulphates*; Appendix 2.3.7.

Tarry matter – Dilute 5 ml with 10 ml of *water*, mix with 6 g of powdered *citric acid* in a small flask, and rotate until dissolved; no tarry or unpleasant odour is perceptible.

Non-volatile residue –Evaporate 50 ml to dryness in a tared porcelain dish and dry to constant weight at 105º, not more than 5 mg of residue remains.

Assay –Weigh accurately about 3 g in flask containing 50 ml of *N sulphuric acid* and titrate the excess of acid with *N sodium hydroxide*, using *methyl red solution* as indicator. Each ml of *N sulphuric acid* is equivalent to 0.01703 g of NH_3.

Storage –Preserve strong Ammonia Solution in a well-closed container, in a cool place.

Ammonia Solution, Iron-free –Dilute ammonia solution which complies with the following additional test :-

Evaporate 5 ml nearly to dryness on a water-bath add 40 ml of water, 2 ml of 20 per cent w/v *solution of iron free citric acid* and 2 drops of *thioglycollic acid*, mix, make alkaline with *iron-free ammonia solution and dilute* to 50 ml with *water*, no pink colour is produced.

Ammonia Buffer pH 10.00 –Ammonia buffer solution. Dissolve 5.4 g of *ammonium chloride* in 70 ml of 5 *N ammonia and dilute* with *water* to 100 ml.

Ammonium Chloride –NH_4Cl = 53.49

Description – Colourless crystals or white crystalline powder; odourless; taste, saline.

Solubility – Freely soluble in *water*, sparingly soluble in alcohol.

Arsenic – Not more than 4 parts per million, Appendix 2.3.1.

Heavy metals –Not more than 10 parts per million, determined by method A, on 2.0 g dissolved in 25 ml of water, Appendix 2.3.3.

Barium – Dissolve 0.5 g in 10 ml of *water* and add 1 ml of *dilute sulphuric acid*; no turbidity is produced within two hours.

Sulphate – 2 g complies with the limit test for sulphates, Appendix 2.3.7

Thiocyanate – Acidify 10 ml of a 10 per cent w/v solution with *hydrochloric acid* and add a few drops of *ferric chloride solution*; no red colour is produced.

Sulphated ash – Not more than 0.1 per cent, Appendix 2.3.6.

Assay – Weigh accurately about 0.1 g, dissolve in 20 ml of water and add a mixture of 5 ml of *formaldehyde solution*, previously neutralised to *dilute phenolphthalein solution* and 20 ml of water. After two minutes, titrate slowly with 0.1 *N sodium hydroxide*, using a further 0.2 ml of *dilute phenolphthalein solution*. Each ml of 0.1N *sodium hydroxide* is equivalent to 0.005349 g of NH_4Cl.

Ammonium Chloride Solution –A 10.0 per cent w/v solution of *ammonium chloride* in water.

Ammonium Citrate Solution –Dissolve with cooling, 500 g *citric acid* in a mixture of 200 ml of *water* and 200 ml of 13.5 M ammonia, filter and dilute with water to 1000 ml.

Ammonium Nitrate – NH_4NO_3 =80.04

Description – Colourless crystals

Solubility – Freely soluble in water

Acidity – A solution in water is slightly acid to litmus *solution*.

Chloride – 3.5 g complies with the limit test for chloride, Appendix 2.3.2.

Sulphate – 5 g complies with the limit test for sulphates, Appendix 2.3.7.

Sulphated ash – Not more than 0.05 per cent, Appendix 2.3.6.

Ammonium Oxalate – $(CO_2NH_4)_2$. H_2O =142.11.

Description –Colourless crystals

Solubility – Soluble in water

Chloride –2 g, with an additional 20 ml of *dilute nitric acid,* complies with the limit test for chlorides, Appendix 2.3.2.

Sulphate –Dissolve 1 g in 50 ml of water, add 2.5 ml of hydrochloric acid and 1ml of *barium chloride solution* and allow to stand for one hour; no turbidity or precipitate is produced.

Sulphated ash – Not more than 0.005 percent, Appendix 2.3.6.

Ammonium Oxalate Solution –A 2.5 per cent w/v solution of *ammonium oxalate* in water.

Ammonium Phosphate – $(NH_4)_2HPO_4$ –

Description –White crystals or granules.

Solubility –Very soluble in wate; insoluble in alcohol.

Reaction – 1 g dissolved in 100 ml of *carbon dioxide-free water* has a reaction of about pH 8.0, using solution of cresol red as indicator.

Iron –2 g complies with the limit test for iron, Appendix 2.3.4.

Chloride – 2 g with an additional 3.5 ml of nitric acid complies with the limit test for chlorides, Appendix 2.3.2.

Sulphate –2.5 g with an additional 4ml of hydrochloric acid, complies with the limit test for sulphate, Appendix 2.3.2.

Ammonium Phosphate, Solution –A 10.0 per cent w/v solution of ammonium phosphate in water.

Ammonium Thiocyanate – NH_4SCN = 76.12.

Description –Colourless crystals.

Solubility – Very soluble in water, forming a clear solution, readily soluble in alcohol.

Chloride –Dissolve 1 g in 30 ml of solution of hydrogen peroxide, add 1 g of *sodium hydroxide*, warm gently, rotate the flask until a vigorous reaction commences and allow to stand until the reaction is complete; add a further 30 ml of *hydrogen peroxide solution* boil for two minutes, cool, and add 10 ml of *dilute nitric acid* and 1 ml of *silver nitrate solution*; any opalescence produced is not greater than that obtained by treating 0.2 ml of 0.01 *N hydrochloric acid* in the same manner.

Sulphated ash –Moisten 1 g with *sulphuric acid* and ignite gently, again moisten with sulphuric acid and ignite; the residue weighs not more than 2.0 mg.

Ammonium Thiocyanate, 0.1N – NH_4SCN = 76.12; 7.612 in 1000 ml. Dissolve about 8 g of *ammonium thiocyanate* in 100o ml of water and standardise the solution as follows :

Pipette 30 ml of standardised 0.1 *N silver nitrate* into a glass stoppered flask, dilute with 50 ml of *water* then add 2 ml of *nitric acid* and 2 ml of *ferric ammonium sulphate solution* and titrate with the *ammonium thiocyanate solution* to the first appearance of a red brown colour. Each ml of 0.1N silver nitrate is equivalent to 0.007612 g of NH_4SCN.

Ammonium Thiocyanate Solution – A 10.0 per cent w/v solution of *ammonium thiocyanate solution*.

Anisaldehyde-Sulphuric Acid Reagent – 0.5 ml *anisaldehyde* is mixed with 10 ml *glacial acetic acid*, followed by 85 ml methanol and 5 ml concentrated *sulphuric acid* in that order.

The reagent has only limited stability and is no longer usable when the colour has turned to redviolet.

Arsenic Trioxide –As_2O_3 = 197.82. Contains not less than 99.8 per cent of As_2O_3.

Description – Heavy white powder

Solubility –Sparingly soluble in water; more readily soluble in water on the addition of *hydrochloric acid*, or solutions of alkali hydroxides or carbonates.

Arsenious sulphide – Weigh accurately 0.50 g and dissolve in 10 ml of *dilute ammonia sulution;* forms a clear colourless solution which, when diluted with an equal volume of water and acidified with *hydrochloric acid*, does not become yellow.

Non-volatile matter –Leaves not more than 0.1 per cent of residue when volatilised.

Assay –Weigh accurately about 0.2 g and dissolve in 20 ml of boiling water and 5 ml of *N sodium hydroxide*, cool, and 5 ml of *N hydrochloric acid* and 3 g of *sodium bicarbonate,* and titrate with 0.1 *N iodine.* Each ml of 0.1N iodine is equivalent to 0.004946 g of As_2O_3.

Barium Chloride - $BaCl_2$, $2H_2O$ =244.27.

Description – Colourless crystals.

Solubility –Freely soluble in water.

Lead –Dissolve 1 g in 40 ml of recently boiled and cooled water, add 5 ml of *lead free acetic acid*. Render alkaline with *lead-free ammonia solution* and add 2 drops of *lead-free sodium sulphide solution;* not more than a slight colour is produced.

Nitrate –Dissolve 1 g in 10 ml of *water*, add 1 ml of *indigo carmine solution* and 10 ml of *nitrogen free sulphuric acid* and heat to boiling; the blue colour does not entirely disappear.

Barium Chloride Solution –A 10.0 per cent w/v solution of *barium chloride* in *water*.

Bismuth Oxynitrate – Bismuth Oxide Nitrate, Contains 70.0 to 74.0 per cent of Bi.

Description –White, microcrystalline powder.

Solubility –Practically insoluble in water, in alcohol; freely soluble in *dilute nitric acid* and in *dilute hydrochloric acid*.

Assay –Weigh accurately about 1 g and dissolve in a mixture of 20 ml of *glycerin* and 20 ml of water. Add 0.1 g of s*ulphamic acid* and titrate with 0.05 M *disodium ethylenediamine tetraacetate*, using *catechol violet solution* as indicator. Each ml of 0.05 M disodium ethylenediamine tetra-acetate is equivalent to 0.01045 g of Bi.

Borax -Sodium Tetraborate , $Na_2B_4O_7$. $10H_2O$ = 381.37. Contains not less than 99.0 per cent and not more than the equivalent of 103.0 per cent of $Na_2B_4O_7$. $10H_2O$.

Description –Transparent, colourless crystals, or a white, crystalline powder; odourless, taste, saline and alkaline. Effloreces in dry air, and on ignition, loses all its water of crystallisation.

Solubility –Soluble in water, practically insoluble in alcohol.

Alkalinity –A solution is alkaline to litmus solution.

Heavy metals –Dissolve 1 g in 16 ml of water and 6 ml of *N hydrochloric acid* and add *water* to make 25 ml; the limit of heavy metals is 20 parts per million, Appendix 2.3.3.

Iron –0.5 g complies with the *limit test for iron*, Appendix 2.3.4

Chlorides –1 g complies with the *limit test for chlorides,* Appendix 2.3.2

Sulphates –1g complies with the *limit test for sulphates,* Appendix 2.3.7.

Assay –Weigh accurately about 3 g and dissolve in 75 ml of *water* and titrate with 0.5 *N hydrochloric acid*, using *methyl red solution* as indicator. Each ml of 0.5 *N* hydrochloric acid is equivalent to 0.09534 g of $Na_2B_4O_7.10.H_2O$.

Storage – Preserve Borax in well-closed container.

Boric Acid –H_3BO_3 = 61.83.

Description –Colourless plates or white crystals or white crystalline powder, greasy to touch; odourless; taste, slightly acid and bitter with a sweetish after taste.

Solubility –Soluble in *water* and in *alcohol*; freely soluble in boiling *water*, in boiling *alcohol* and in *glycerin.*

Sulphate –Boil 3 g with 30 ml of *water* and 1 ml of *hydrochloric acid*, cool, and filter; 25 ml of the filtrate complies with the *limit test for sulphates*, Appendix 2.3.7.

Arsenic –Not more than 10 parts per million, Appendix 2.3.1.

Heavy metals –Not more than 20 parts per million, determined by Method A on a solution obtained by dissolving 1.0 g in 2 ml of *dilute acetic acid* and sufficient *water* to produce 25 ml, Appendix 2.3.3.

Assay –Weigh accurately about 2 g, and dissolve in a mixture of 50 ml of *water* and 100 ml of *glycerine,* previously neutralised to *phenolphthalein solution*. Titrate with *N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.06183 g of H_3BO_3.

Storage –Store in well-closed containers.

Labelling –The label on the container states "Not for internal use".

Boric Acid Solution

–Dissolve 5 g of boric acid in a mixture of 20 ml of *water* and 20 ml of *absolute ethanol* and dilute with *absolute ethanol* to 250 ml.

Bromine – Br2 =159.80.

Description –Reddish-brown, fuming, corrosive liquid.

Solubility –Slightly soluble in *water*, soluble in most organic solvents.

Iodine –Boil 0.2 ml with 20 ml of *water*, 0.2 ml of *N sulphuric acid* and a small piece of marble until the liquid is almost colourless. Cool, add one drop of *liquified phenol*, allow to stand for two minutes, and then add 0.2 g of *potassium iodide* and 1 ml of *starch solution*; no blue colour is produced.

Sulphate –Shake 3 ml with 30 ml of *dilute ammonia solution* and evaporate to dryness on a water bath, the residue complies with the *limit test for sulphates*, Appendix 2.3.7.

Bromine Solution – Dissolve 9.6 ml of *bromine* and 30 g of *potassium bromide* in sufficient *water* to produce 100 ml.

Bromocresol Purple – 4,4' –(3H-2, 1-Benzoxathiol-3-ylidene) bis-(2,6-dibromo-o-cresol) SS-dioxide; $C_{21}H_{14}Br_2O_4S$ =540.2.

Gives a yellow colour in weakly acid solutions and a bluish-violet colour in alkaline, neutral and extremely weakly acid solutions (pH range, 5.2 to 6.8).

Bromocresol Purple Solution –Warm 0.1 g of *bromocresol purple* with 5 ml of *ethanol* (90 per cent) until dissolved, add 100 ml of ethanol (20 per cent), 3.7 ml of 0.05 M s*odium hydroxide*, and sufficient *ethanol (20 per cent)* to produce 250 ml.

Complies with the following test :

Sensitivity –A mixture of 0.2 ml of the solution and 100 ml of *carbon dioxide-free water* to which 0.05 ml of 0.02 M *sodium hydroxide* has been added is bluish-violet. Not more than 0.20 ml of 0.02 M *hydrochloric acid* is required to change the colour to yellow.

Bromophenol Blue –4, 4/, -(3H-2, 1-Benzoxathiol-3-ylidene) bis-(2-6-dibromophenol) SS-dioxide C19H19Br4O5S = 670.

Gives a yellow colour in moderately acid solutions, and a bluish-violet colour in weakly acid and alkaline solutions (pH range, 2.8 to 4.6).

Bromophenol Blue Solution – Warm 0.1 g of *bromophenol blue* with 3.0 ml of 0.05 *N sodium hydroxide* and 5 ml of *alcohol* (90 per cent); after solution is effected, add sufficient *alcohol* (20 per cent) to produce 250 ml.

Complies with the following test :

Sensitivity –A mixture of 0.05 ml of the solution and 20 ml of *carbon dioxide-free water* to which 0.05 ml of 0.1*N hydrochloric acid* has been added is yellow. Not more than 0.10 ml of 0.1 *N sodium hydroxide* is required to change the colour to bluish-violet.

Bromothymol Blue –6, 6/–(3H-2, 1–Benzoxathiol–3–ylidene) bis –(2 –bromothymol) SS–dioxide $C_{27}H_{28}Br_2O_5S$ = 624.

Gives a yellow colour in weakly acid solutions and a blue colour in weakly alkaline solutions. Neutrality is indicated by a green colour (pH range, 6.0 to 7.6).

Bromothymol Blue Solution –Warm 0.1 g of *bromothymol blue* with 3.2 ml of 0.05 *N sodium hydroxide* and 5 ml of alcohol (90 per cent); after solution is effected, add sufficient alcohol (20 per cent) to produce 250 ml.

Complies with the following test :

Sensitivity –A mixture to 0.3 ml of the solution and 100 ml of c*arbon dioxide-free water* is yellow. Not more than 0.10 ml of 0.02 *N sodium hydroxide* is required to change the colour to blue.

Cadmium Iodide – CdI_2 = 366.23

Description –Pearly white flakes or a crystalline powder.

Solubility –Freely soluble in water.

Iodate –Dissolve 0.2 g in 10 ml of *water*, and add 0.5 g of *citric acid* and 1 ml of *starch solution*, no blue colour is produced.

Cadmium Iodide Solution – A 5.0 per cent w/v solution of *cadmium iodide* in *water*.

Calcium Carbonate – $CaCO_3$ = 100 .1

Analytical reagent grade of commerce.

Calcium Chloride – $CaCl_2$ H_2O = 147.0.

Analytical reagent grade of commerce.

Calcium Chloride Solution –A 10 per cent w/v solution of calcium chloride in water.

Calcium Hydroxide – $Ca(OH)_2$ = 74.09

Analytical reagent grade of commerce.

Calcium Hydroxide Solution –Shake 10 g of calcium hydroxide repeatedly with 1000 ml of water and allow to stand until clear.

Calcium Sulphate – $CaSO_4$, $2H_2O$ = 172.17.

Description –White powder.

Solubility –Slightly soluble in *water*.

Chloride –Boil 5 g with 50 ml of *water* and filter while hot. The filtrate, after cooling complies with the limit test for chlorides, Appendix 2.3.2.

Acid-insoluble matter –Boil 2 g with 100 ml of *N hydrochloric acid*; and then with *water*, dry, ignite, and weigh; the residue weighs not more than 2 mg.

Alkalinity –Boil 1 g with 50 ml of water, cool, and titrate with 0.*1 N hydrochloric acid*, *using bromo thymol blue solution* as indicator; not more than 0.3 ml of 0.1 *N hydrochloric acid* is required.

Carbonate –Boil 1 g with 10 ml of *water* and 1 ml of *hydrochloric acid,* no carbon dioxide is evolved.

Residue on ignition –When ignited, leaves not less than 78.5 per cent and not more than 80.0 per cent of residue.

Camphor –C10H16O = 152.23

Camphor is a ketone, obtained from *Cinnamomum camphora* (Linn.) Nees and Eberm. (Fam. Lauraceae) and *Ocimum kilimandscharicum Guerke* (Fam. Labiatae) (Natural Camphor) or produced synthetically (Synthetic Camphor). It contains not less than 96.0 per cent of $C_{10}H_{16}O$.

Description – Colourless or white crystals, granules or crystalline masses or colourless to white, translucent tough masses; odour, penetrating and characteristic; taste, pungent, aromatic, and followed by a sensation of cold. Readily pulverisable in the presence of a little *alcohol, chloroform*, or solvent ether.

Solubility –Slightly soluble in water, very soluble in *alcohol, in chloroform* and in *solvent ether*, freely soluble in fixed oils and in volatile oils.

Melting range –174º to 179º.

Specific optical rotation – + 41º to + 43º, determined in a 10 per cent w/v solution of Natural Camphor in alcohol. Synthetic Camphor is the optically inactive, racemic form.

Water – A 10 per cent w/v solution in light petroleum (boiling range 40° to 60º) is clear.

Non-volatile matter – Leaves not more than 0.05 per cent of residue when volatilised at 105º.

Assay – Weigh accurately about 0.2 g and dissolve in 25 ml of *aldehyde-free alcohol*, in a 300-ml flask. Slowly add while stirring 75 ml of *dinitrophenylhydrazine* solution and heat on a water-bath for four hours under a reflux condenser. Remove the alcohol by distillation, allow to cool, dilute to 200 ml with a 2 per cent v/v solution of *sulphuric acid* in water. Set aside for twenty-four hours, filter in a tared Gooch crucible, and wash the precipitate with successive quantities of 10 ml of cold water until the washings are neutral to *litmus paper*. Dry to constant weight at 80º and weigh. Each g of precipitate is equivalent to 0.458 g of C10H16O.

Storage –Preserve Camphor in a well-closed container in a cool place.

Canada Balsam Reagent –General reagent grade of commerce.

Carbon Dioxide – CO_2 = 44. 01.

Commercially available carbon dioxide.

Carbon Disulphide – CS_2 = 76.14

Description –Clear, almost colourless, flammable liquid.

Distillation range – Not less than 95 per cent distils between 46º and 47º.

Wt. per ml – At 25º, about 1.263 g.

Non-volatile matter –When evaporated to dryness on a water bath, and dried to constant weight at 105º. Leaves not more than 0.005 per cent w/v of residue.

Carbon Tetrachloride – CCl_4 = 153.82

Description –Clear, colourless, volatile, liquid; odour, characteristic.

Solubility –Practically insoluble in water; miscible with ethyl alcohol, and with solvent ether.

Distillation range –Not less than 95 per cent distils between 76º and 77º.

Wt per ml – At 20º, 1.592 to 1.595 g.

Chloride, Free acid –Shake 20 ml with 20 ml of freshly boiled and cooled water for three minutes and allow separation to take place; the aqueous layer complies with the following test :

Chloride – To 10 ml add one drop of nitric acid and 0.2 ml of *silver nitrate solution*; no opalescence is produced.

Free acid –To 10 ml add a few drops of *bromocresol purple solution;* the colour produced does not indicate more acidity than that indicated by the addition of the same quantity of the indicator to 10 ml of freshly boiled and cooled *water.*

Free chlorine –Shake 10 ml with 5 ml of *cadmium iodide solution* and 1 ml *of starch solution*, no blue colour is produced.

Oxidisable impurities –Shake 20 ml for five minutes with a cold mixture of 10 ml of *sulphuric acid* and 10 ml of 0.1 *N potassium dichromate*, dilute with 100 ml of water and add 3 g of *potassium iodide* : the liberated iodine requires for decolourisation not less than 9 ml of 0.1 *N sodium thiosulphate*.

Non-volatile matter –Leaves on evaporation on a water-bath and drying to constant weight at 105º not more than 0.002 per cent w/v of residue.

Caustic Alkali Solution, 5 per cent –

Dissolve 5 g of *potassium or sodium hydroxide* in *water* and dilute to 100 ml.

Charcoal, Decolourising –General purpose grade complying with the following test.

Decolourising powder –Add 0.10 g to 50 ml of 0.006 per cent w/v solution of *bromophenol blue* in ethanol (20 per cent) contained in a 250 ml flask, and mix. Allow to stand for five minutes, and filter; the colour of the filterate is not deeper than that of a solution prepared by diluting 1 ml of the *bromophenol blue solution* with *ethanol* (20 per cent) to 50 ml.

Chloral Hydrate –$CCl_3.CH(OH)_2$ = 165.40.

Description –Colourless, transparent crystals, odour, pungent but not acrid; taste, pungent and slightly bitter, volatilises slowly on exposure to air.

Solubility –Very soluble in *water,* freely soluble in *alcohol*, in chloroform and in *solvent ether*.

Chloral alcoholate – Warm 1 g with 6 ml of *water* and 0.5 ml of *sodium hydroxide solution* : filter, add sufficient 0*.1 N iodine* to impart a deep brown colour, and set aside for one hour; no yellow crystalline precipitate is produced and no smell of iodoform is perceptible.

Chloride – 3 g complies with the limit test for chlorides, Appendix 2.3.2.

Assay – Weigh accurately about 4 g and dissolve in 10 ml of *water* and add 30 ml of *N sodium hydroxide.* Allow the mixture to stand for two minutes, and then titrate with *N sulphuric acid* using *phenolphthalein solution* as indicator. Titrate the neutralised liquid with 0.1 *N silver nitrate* using solution of *potassium chromate* as indicator. Add two-fifteenth of the amount of 0.1 *N silver nitrate* used to the amount of *N sulphuric acid* used in the first titration and deduct the figure so obtained from the amount of *N sodium hydroxide* added. Each ml of *N sodium hydroxide,* obtained as difference; is equivalent to 0.1654 g of $C_2H_3Cl_3O_2$.

Storage – Store in tightly closed, light resistant containers in a cool place.

Chloral Hydrate Solution –Dissolve 20 g of *chloral hydrate* in 5 ml of water with warming and add 5 ml of *glycerin*.

Chloral Iodine Solution –Add an excess of crystalline *iodine* with shaking to the *chloral hydrate solution*, so that crystals of undissolved iodine remain on the bottom of bottle. Shake before use as the iodine dissolves, and crystals of the iodine to the solution. Store in a bottle of amber glass in a place protected from light.

Chlorinated Lime –Bleaching powder. Contains not less than 3.0 per cent of available chlorine.

Description –A dull white powder; odour characteristic. On exposure to air it becomes moist and gradually decomposes.

Solubility –Slightly soluble in water and in alcohol.

Stability –Loses not more than 3.0 per cent of its available chlorine by weight when heated to 100º for two hours (The available chlorine is determined by the Assay described below).

Assay –Weigh accurately about 4 g, triturate in a mortar with successive small quantities of water and transfer to a 1000 ml flask. Add sufficient water to produce 1000 ml and shake thoroughly. To 100 ml to this suspension add 3 g of *potassium iodide* dissolved in 100 ml of *water*, acidify with 5 ml of *acetic acid* and titrate the liberated iodine with 0.1 *N sodium thiosulphate*. Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.003545 g of available chlorine.

Storage –Preserve in a well-closed container.

Chlorinated Lime Solution. –Mix 100 g of *chlorinated lime* with 1000 ml of *water;* transfer the mixture to a stoppered bottle; set aside for three hours, shaking occasionally; filter through calico.

Chlorinated lime solution must be recently prepared.

Chloroform – $CHCl_3$ = 119.38

Description –Colourles, volatile liquid; odour, characteristic. taste, sweet and burning.

Solubility –Slightly soluble in water; freely miscible with ethyl alcohol and with solvent ether.

Wt. Per ml. : Between 1.474 and 1.478 g.

Boiling range – A variable fraction, not exceeding 5 per cent v/v, distils below 60º and the remainder distils between 50º to 62º.

Acidity –Shake 10 ml with 20 ml of freshly boiled and cooled water for three minutes, and allow to separate. To a 5 ml portion of the aqueous layer add 0.1 ml of *litmus solution*; the colour produced is not different from that produced on adding 0.1 ml of *litmus solution* to 5 ml of freshly boiled and cooled water.

Chloride –To another 5 ml portion of the aqueous layer obtained in the test for Acidity, add 5 ml of water and 0.2 ml of *silver nitrate solution*; no opalescence is produced.

Free chlorine –To another 10 ml portion of the aqueous layer, obtained in the test for Acidity, add 1 ml of *cadmium iodide solution* and two drops of starch solution; no blue colour is produced.

Aldehyde –Shake 5 ml with 5 ml of water and 0.2 ml of *alkaline potassium mercuri-iodide solution* in a stoppered bottle and set aside in the dark for fifteen minutes; not more than a pale yellow colour is produced.

Decomposition products – Place 20 ml of the *chloroform* in a glass-stoppered flask, previously rinsed with *sulphuric acid,* add 15 ml of *sulphuric acid* and four drops of *formaldehyde solution*, and shake the mixture frequently during half an hour and set aside for further half an hour, the flask being protected from light during the test; the acid layer is not more than slightly coloured.

Foreign organic matter – Shake 20 ml with 10 ml of *sulphuric* acid in a stoppered vessel previously rinsed with *sulphuric acid* for five minutes and set aside in the dark for thirty minutes, both the acid and chloroform layers remain colourless. To 2 ml of the acid layer add 5 ml of water; the liquid remains colourless and clear, and has no unpleasent odour. Add a further 10 ml of water and 0.2 ml of *silver nitrate solution*; no opalescence is produced.

Foreign odour –Allow 10 ml to evaporate from a large piece of filter paper placed on a warm plate; no foreign odour is detectable at any stage of the evaporation.

Non volatile matter – Not more than 0.004 per cent w/v determined on 25 ml by evaporation and drying at 105º.

Storage : Store in tightly-closed, glass-stoppered, light-resistant bottles.

Note :- Care should be taken not to vaporise Chloroform in the presence of a flame because of the production of harmful gases.

Chloroform Water –

Chloroform	:	2.5 ml
Purified Water	:	sufficient to produce 1000 ml

Dissolve the C*hloroform* in the purified water by shaking.

Chromic-Sulphuric Acid Mixture –A saturated solution of Chromium trioxide in sulphuric acid .

Chromium Trioxide – CrO_3 = 99.99

Analytical reagent grade.

Chromotropic Acid – $C_{10}H_8O_8S_2.2H_2O$ = 356.32

Description –White to brownish powder. It is usually available as its sodium salt, $C_{10}H_8O_8S_2Na_2$, which is yellow to light brown in colour.

Solubility –Soluble in water; sodium salt is freely soluble in water.

Sensitivity –Dilute exactly 0.5 ml *formaldehyde solution* with water to make 1000 ml. Disslove 5 mg of *chromotropic acid* or its sodium salt, in a 10 ml of a mixture of 9 ml *of sulphuric acid* and 4 ml of water. Add 5 ml of this solution to 0.2 ml of the *formaldehyde solution*, and heat for 10 minutes at 60º; a violet colour is produced.

Chromotropic Acid Solution –Dissolve 5 mg of *chromotropic acid sodium* salt in 10 ml of a mixture of 9 ml of *sulphuric acid* and 4 ml of water.

Citric Acid – $C_6H_8O_7$, H_2O =210.1

Colourless, translucent crystals, or a white, crystalline powder, slightly hygroscopic in moist air and slightly efflorescent in warm dry air; odourless; taste, strongly acid.

Analytical reagent grade.

Citric Acid, Iron-Free –Citric acid which complies following additional test :

Dissolve 0.5 g in 40 ml of water, add 2 drops of thioglycollic acid, mix, make alkaline with iron free ammonia solution and dilute to 50 ml with water; no pink colour is produced.

Copper Acetate –$Cu(C_2H_3O_2)_2$, H_2O =199.65

Contains not less than 98.0 per cent of $C_4H_6O_4Cu$, H_2O

Description –Blue-green crystals or powder, having a faint odour of acetic acid.

Solubility – Soluble in water, yielding a clear solution.

Chloride –3g complies with the *limit test for chlorides*, Appendix 2.3.2.

Sulphate –3g complies with the *limit test for sulphates*, Appendix 2.3.7.

Assay –Weigh accurately about 0.8 g and dissolve in 50 ml of water, add 2 ml of *acetic acid* and 3 g of *potassium iodide*, and titrate the liberated iodine with 0.1 *N sodium thiosulphate*, using starch solution as indicator, until only a faint blue colour remains; add 2 g of *potassium thiocyanate* and continue the titration until the blue colour disappears. Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.01997 g of $C_4H_6O_4Cu$, H_2O

Copper Acetate, Solution –0.5 per cent w/v of copper acetate in water.

Copper Sulphate – $CuSO_4$, $5H_2O$ = 249.68

Contains not less than 98.5 per cent and not more than the equivalent of 101.0 per cent of $CuSO_4$, $5H_2O$.

Description –Blue triclinic prisms or a blue, crystalline powder.

Solubility –Soluble in *water*, very solube in boiling water, almost insoluble in *alcohol*; very slowly soluble in *glycerin.*

Acidity and clarity of solution – 1 g, dissolved in 20 ml of water,forms a clear blue solution, which becomes green on the addition of 0.1 ml of *methyl orange solution*.

Iron – To 5 g, add 25 ml of water, and 2 ml of nitric acid, boil and cool. Add excess of *strong ammonia solution*, filter, and wash the residue with *dilute ammonia solution* mixed with four times its volumes of water. Dissolve the residue, if any, on the filter with 2 ml of *hydrochloric acid*, diluted with 10 ml of water; to the acid solutions add *dilute ammonia solution* till the precipitation is complete; filter and wash; the residue after ignition weighs not more than 7 mg.

Copper Sulphate, Anhydrous –$CuSO_4$ =159.6

Prepared by heating copper sulphate to constant weight at about 230º.

Copper Sulphate Solution –A10.0 per cent w/v solution of *copper sulphate* in *water*.

Catechol Violet – 4,4/ –(3H-2, I-Benzoxathiol-3-ylidene) diphyrocatechol SS-dioxide.

Gives a blue colour with bishmuth ions in moderately acid solution. When metal ions are absent, for example, in the presence of an excess of *disodium ethylenediamine tetra-acetate*, the solution is yellow.

Catechol Violet Solution –Dissolve 0.1 g of catechol violet in 100 ml of water.

Cresol Red – 4,4$^{/}$, –(3H-2, 1-Benzoxathiol-3 ylidene) di-o-cresol SS-dioxide; $C_{12}H_8O_5S$ = 382.4.

Gives a red colour in very strongly acid solutions, a yellow colour in less strongly acid and neutral solutions, and a red colour in moderately alkaline solutions (pH ranges, 0.2 to 1.8, and 7.2 to 8.8).

Cresol Red Solution –Warm 50 ml of *cresol red* with 2.65 ml of 0.05 M s*odium hydroxide* and 5 ml of *ethanol (90 per cent);* after solution is effected, add sufficient ethanol (20 per cent) to produce 250 ml.

Sensitivity –A mixitue of 0.1 ml of the solution and 100 ml of *carbon dioxide-free water* to which 0.15 ml of 0.02 M s*odium hydroxide* has been added is purplish-red.Not more than 0.15 ml of 0.02 *M hydrochloric acid* is required to change the colour to yellow.

Dimethyl Yellow – 4 –Dimethyl aminoazobenzene ; $C_{14}H_{15}5N_3$ = 225.3

Gives a red colour in moderately acid alcoholic solutions, and a yellow colour in weakly acid and alkaline solution (pH range, 2.8 to 4.0).

Dimethyl Yellow Solution –A 0.2 per cent w/v solution of *dimethyl yellow* in alcohol (90 per cent).

Sensitivity – A solution containing 2 g of ammonium chloride in 25 ml of *carbon dioxide-free water,* to which is added 0.1 ml of the *dimethyl yellow solution*, is yellow. Not more than 0.10 ml of 0.1 *N hydrochloric acid* is required to change the colour to red.

Dinitrophenylhydrazine –2,4–Dinitrophenylhydrazine; $(NO_2)_2C_6H_3$, NH, NH_3 = 198.14.

Description –Orange-red crystals or a crystalline powder.

Solubility –Practically insoluble in *water,* slightly soluble in alcohol.

Clarity and colour of solution – 0.5 g yields a clear yellow solution on heating with a mixture of 25 ml of water and 25 ml of h*ydrochloric acid*.

Melting range –197º to 200º, with decomposition.

Sulphated ash –Not more than 0.5 per cent, Appendix 2.3.6.

Dinitrophenylhydrazine Solution –Dissolve 1.5 gm of *dinitrophenylhydrazine* in 20 ml of sulphuric acid (50 per cent v/v). Dilute to 100 ml with *water* and filter.

Dinitrophenylhydrazine solution must be freshly prepared.

Diphenylbenzidine –$(C_6H_5.\ NH.\ C_6H_4)_2$ = 336.42.

Description – White for faintly grey coloured, crystalline powder.

Melting range –246º to 250º.

Nitrate –Dissolve 8 mg in a cooled mixture of 45 ml of *nitrogen free sulphuric acid* and 5 ml of water; the solution is colourless or not more than very pale blue.

Sulphated ash –Not more than 0.1 per cent, Appendix 2.3.6.

Diphenylcarbazide –1,5-Diphenylcarbazide : $(C_6H_5NH.\ NH)_2\ CO$ = 242.27.

Description –White crystalline powder which gradually acquires a pink tint on exposure to air.

Solubility –Practically insoluble in *water*; soluble in alcohol.

Diphenylcarbazide Solution –A 0.2 per cent w/v solution of *diphenylcarbazide* in a mixture of 10 ml of glacial acetic acid and 90 ml of *alcohol (90 per cent).*

Diphenylthiocarbazone –Dithizone : 1,5–Diphenylthiocarbazone; $C_6H_5N : NCS.\ NH.\ NH.\ C_6H_5$ = 256.32.

Description –Almost black powder.

Solubility –Practically insoluble in *water*; soluble in *chloroform*, in carbon tetrachloride and in other organic solvents, yielding solutions of an intense green colour.

Lead –Shake 5 ml of 0.1 per cent w/v solution in *chloroform* with a mixture of 5 ml of *water*, 2 ml of *lead free potassium cyanide solution,* and 5 ml of *strong ammonia solution*; the chloroform layer may remain yellow but has no red tint.

Sulphated ash –Not more than 0.5 per cent, Appendix 2.3.6.

Disodium Ethylenediamine tetraacetate –(Disodium Acetate)
$C_{10}H_{14}N_2Na_2O_8, 2H_2O = 372.2$

Analytical reagent grade.

Dragendorff Reagent –

Solution 1 –Dissolve 0.85 g of *bismuth oxy nitrate* in 40 ml of water and 10 ml of acetic acid.

Solution 2 –Dissolve 8 g of *potassium iodide* in 20 ml of water.

Mix equal volumes of solution 1 and 2, and to 10 ml of the resultant mixture add 100 ml of water and 20 ml of acetic acid.

Eosin – Acid Red 87; Tetrabromofluorescein disodium salt; $C_{20}H_6O_5Br_4Na_2$ =691.86.

Description – Red powder, dissolves in water to yield a yellow to *purplish-red* solution with a greenish-yellow fluorescence.

Solubility –Soluble in *water* and in alcohol.

Chloride –Dissolve 50 mg in 25 ml of *water*, add 1 ml of *nitric acid*, and filter; the filtrate complies with *the limit test for chlorides*, Appendix 2.3.2.

Sulphated ash –Not more than 24.0 per cent, calculated with reference to the substance dried at 110º for two hours, Appendix 2.3.6.

Eosin Solution –A 0.5 per cent w/v solution of eosin in water.

Eriochrome Black T –Mordant Black 11; Sodium 2(1-hydroxy-2-naphthylazo) 5-nitro-2-naphtol-4-sulphonate; $C_{20}H_{12}N_3NaO_7S$ = 461.38.

Brownish black powder having a faint, metallic sheen, soluble in alcohol, in *methyl alcohol* and in hot water.

Ether, Diethyl Ether – $(C_2H_5)_2O$ = 74.12.

Analytical reagent grade.

A volatile, highly flammable, colourless liquid, boiling point, about 34º; weight per ml about 0.71g .

Warning –It is dangerous to distil or evaporate ether to dryness unless precautions have been taken to remove peroxides.

Ethyl Acetate –$CH_3. CO_2C_2H_5$ = 88.11.

Analytical reagent grade.

A colourless liquid with a fruity odour; boiling point, about 77º; weight per ml about 0.90g.

Ethyl Alcohol –C_2H_5OH = 46.07.

Absolute Alcohol; Dehydrated Alcohol.

Description –Clear, colourless, mobile, volatile liquid; odour, characteristic and spirituous; taste, burning; hygroscopic. Readily volatilisable even at low temperature and boils at 78º and is flammable.

Solubility –Miscible with water, with solvent ether and with chloroform.

Contains not less than 99.5 per cent w/w or 99.7 per cent v/v of C_2H_5OH..

Identification –Acidity or Alkalinity : Clarity of Solution; Methanol; Foreign organic substances; Isopropyl alcohol and butyl alcohol; Aldehydes and ketones; fusel oil constituents; Non-volatile matter; complies with the requirements described under Alcohol.

Specific gravity –Between 0.7871 and 0.7902, at 25º.

Storage –Store in tightly closed containers in a cool place away from fire and protected from moisture.

Labelling –The label on the container states "Flammable".

Ferric Ammonium Sulphate –Ferric Alum, $Fe (NH_4) (SO_4)_2, 12H_2O$ = 482.18

Contains not less than 99.0 per cent and not more than the equivalent of 101.0 per cent of $Fe(NH_4) (SO_4)_2$, 12 H_2O.

Description –Pale violet crystals, or a nearly colourless crystalline powder.

Solubility –Soluble in water, yielding a clear yellow or brown solution.

Ferrous iron –Dissolve 1 g in 50 ml of *water*, add 1 ml of *dilute hydrochloric acid* and 1 ml of *potassium ferricyanide solution*; no green or blue colour is produced.

Assay –Weigh accurately about 2 g, dissolve in 10 ml of *dilute hydrochloric acid* and dilute to 50 ml with water, add 3 g of *potassium iodide*, allow to stand for ten minutes titrate the liberated iodine with 0.1 *N sodium thiosulphate*, using starch solution as indicator added towards the end of titrations. Each. ml of 0.1 *N sodium thiosulphate* is equivalent to 0.04822 g of $Fe(NH_4) (SO_4)_2$. $12H_2O$.

Ferric Ammonium Sulphate 0.1N – $FeNH_4(SO_4)_2, 12H_2O$ = 482.18; 48,22 g in 1000 ml.

Dissolve 50 g of *ferric-ammonium sulphate* in a mixture of 300 ml of water and 6 ml of *sulphuric acid,* dilute with water to 1000 ml, and mix. Standardise the solution as follows :-

Measure accurately about 30 ml of the solution into a glass-stoppered flask, add 5 ml of *hydrochloric acid*, mix, and add a solution of 3 g of *potassium iodide* in 10 ml of *water*. Insert the stopper, allow to stand for ten minutes in the dark, then titrate the liberated iodine with standardised 0.*1N sodium thiosulophate*, adding 3 ml of *starch solution* as the end-point is approached. Perform a blank determination and make any necessary correction. Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.04822 g of $FeNH_4(SO_4)_2$, $12H_2O$.

Note –Store 0.1 N Ferric Ammonium Sulphate in tightly-closed, light resistant containers.

Ferric Chloride –Anhydrous Ferric Chloride; $FeCl_3$ = 162.22

Description –Greenish-black crystals or a crystalline powder, free from the orange colour of the hydrated salt, which is readily acquired by exposure to atmospheric moisture.

Solubility –Soluble in *water*, yielding an orange coloured opalescent solution.

Ferrous salts –Dissolve 2.0 g in 100 ml of water, add 2 ml of *phosphoric acid* and titrate with 0.1 *N potassium permanganate* until a pink colour is produced, not more than 0.1 ml is required.

Free chloride –Dissolve 5 g in 10 ml of water and boil the solution; no blue colour is prroduced on a starch iodide paper exposed to the vapours.

Ferric Chloride Solution –Contains not less than 14.25 per cent and not more than 15.75 per cent w/v of $FeCl_3$.

Description –Clear, Yellowish-brown liquid.
Assay –Dilute 2 ml with 20 ml of water, add 1 ml of *sulphuric acid* and 0.*1 N potassium permanganate* drop by drop until a pink colour persists for five seconds. Add 15 ml of *hydrochloric acid* and 2 g of *potassium iodide*, allow to stand for three minutes, and titrate with 0.1 *N sodium thiosulphate*, using starch solution as indicator added towards the end of titration. Each ml of 0.*1 N sodium thiosulphate* is equivalent to 0.01622 g of FeCl3.

Ferrous Sulphate – FeSO4. 7H2O = 278.0
Description –Transparent, green crystals, or a pale bluish-green, crystalline powder; odourless; taste, metallic and astringent, Efflorescent in dry air. On exposure to moist air, the crystals rapidly oxidise and become coated with brownish yellow basic ferrous sulphate.

Solubility –Freely soluble in water, very soluble in boiling water, practically insoluble in alcohol.

pH-Between 3.0 and 4.0, determined in a 5.0 per cent w/v solution.

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.

Copper – Dissolve 2 g in 50 ml of *water*, acidify with 1 ml of *dilute sulphuric acid,* saturate with *solution of hydrogen sulphide;* no darkening or precipitate is produced.

Ferrous Sulphate Solution –A 2.0 per cent w/v solution *of ferrous sulphate* in freshly boiled and cooled water.

Ferrous sulphate solution must be freshly prepared.

Ferrous Sulphate Solution, Acid –A 0.45 per cent w/v solution of *ferrous sulphate* in freshly boiled and cooled *water containing* 0.5 ml of hydrochloric acid.

Formaldehyde Solution –Formalin; HCHO =30.03

Formaldehyde Solution is a solution of formaldehyde in water with *methyl alcohol* added to prevent polymerisation. It contains not less than 34.0 per cent w/w and not more than 38.0 per cent w/w of CH2O.

Description –Colourless liquid; odour, characteristic, pungent and irritating; taste, burning. A slight white cloudy deposit is formed on long standing, especially in the cold, due to the separation of paraformaldehyde. This white deposit disappears on warming the solution.

Solubility –Miscible with *water*, and with *alcohol*.

Acidity –To 10 ml add 10 ml of *carbon dioxide free water* and titrate with 0.1 *N sodium hydroxide* using *bromothymol blue solution* as indicator; not more than 5 ml of 0.1 *N sodium hydroxide* is required.

Wt. per ml – At 20º, 1.079 to 1.094 g.

Assay –Weigh accurately about 3 g and add to a mixture of 50 ml of *hydrogen peroxide solution* and 50 ml of *N sodium hydroxide*, warm on a water-bath until effervescence ceases and titrate the excess of alkali with *N sulphuric acid* using *phenolphthalein solution* as indicator. Repeat the experiment with the same quantities of the same reagents in the same manner omitting the formaldehyde solution. The difference between the titrations represents the sodium hydroxide required to neutralise the formic acid produced by the oxidation of the formaldehyde. Each ml of N sodium hydroxide is equivalent to 0.03003 g of CH_2O.

Storage–Preserve Formaldehyde Solution in well-closed container preferably at a temperature not below 15º

Formaldehyde Solution, Dilute –

Dilute 34 ml of *formaldehyde solution* with sufficient water to produce 100 ml.

Glycerin –$C_3H_8O_3$ = 82.09.

Description – Clear, colourless liquid of syrupy consistency; odourless, taste sweet followed by a sensation of warmth. It is hygroscopic.

Solubility –Miscible with water and with *alcohol*; practically insoluble in chloroform, in solvent ether and in fixed oils.

Acidity –To 50 ml of a 50 per cent w/v solution add 0.2 ml of *dilute phenolphthalein solution*; not more than 0.2 ml of 0.1 *N sodium hydroxide* is required to produce a pink colour.

Wt. per ml –Between 1.252 g and 1.257 g, corresponding to between 98.0 per cent and 100.0 per cent w/w of $C_3H_8O_3$.

Refractive index –Between 1.470 and 1.475 determined at 20º.

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.

Copper –To 10 ml add 30 ml of *water*, and 1 ml of *dilute hydrochloric acid*, and 10 ml of *hydrogen sulphide solution*; no colour is produced.

Iron – 10 g complies with the *limit test* for iron, Appendix 2.3.4.

Heavy metals – Not more than 5 parts per million, determined by Method A on a solution of 4 g in 2 ml of 0.1 *N hydrochloric acid* and sufficient water to produce 25 ml, Appendix 2.3.3.

Sulphate –1 ml complies with the *limit test* for sulphates, Appendix 2.3.7.

Chloride –1 ml complies with the *limit test* for chloride, Appendix 2.3.2.

Acraldehyde and glucose –Heat strongly; it assumes not more than a faint yellow, and not a pink colour. Heat further; it burns with little or no charring and with no odour of burnt sugar.

Aldehydes and related substances – To 12.5 ml of a 50 per cent w/v solution in a glass-stoppered flask add 2.5 ml of *water* and 1 ml of *decolorised magenta solution*. Close the flask and allow to stand for one hour. Any violet colour produced is not more intense than that produced by mixing 1.6 ml of 0.*1 N potassium permanganate* and 250 ml of *water*.

Sugar –Heat 5 g with 1 ml of *dilute sulphuric acid* for five minutes on a water-bath. Add 2 ml of *dilute sodium hydroxide solution* and 1 ml of *copper sulphate solution*. A clear, blue coloured solution is produced. Continue heating on the water-bath for five minutes. The solution remains blue and no precipitate is formed.

Fatty acids and esters –Mix 50 ml with 50 ml of freshly boiled *water* and 50.0 ml of 0.5N *sodium hydroxide,* boil the mixture for five minutes. Cool, add a few drops of *phenolphthalein solution* and titrate the excess alkali with 0.5 *N hydrochloric acid*. Perform a blank determination , not more than 1 ml of 0.5 *N sodium hydroxide* is consumed.

Sulphated ash –Not more than 0.01 per cent, Appendix 2.3.6.

Storage –Store in tightly-closed containers.

Glycerin Solution –Dilute 33 ml of glycerin to 100 ml with water and add a small piece of camphor or liquid phenol.

Hexamine – $(CH_2)_6N_4$ = 140.2

Analytical reagent grade.

Hydrazine Hydrate –NH_2. NH_2. H_2O = 50.06

Analytical reagent grade.

A colourless liquid with an ammonical odour; weight per ml. about 1.03 g.

Hydrochloric Acid –HCl = 36.46

Concentrated Hydrochloric Acid

Description –Clear, colourless, fuming liquid; odour, pungent.

Arsenic –Not more than 1 part per million, Appendix 2.3.1.

Heavy metals –Not more than 5 parts per million, determined by Method A on a solution prepared in the following manner : Evaporate 3.5 ml to dryness on a water-bath, add 2 ml of *dilute acetic acid* to the residue, and add water to make 25 ml, Appendix 2.3.3.

Bromide and iodide –Dilute 5 ml with 10 ml of *water*, add 1 ml of *chloroform*, and add drop by drop, with constant shaking, *chlorinated lime solution*; the chloroform layer does not become brown or violet.

Sulphite –Dilute 1 ml with 10 ml of water, and add 5 drops of *barium chloride solution* and 0.5 ml of 0.001 *N iodine*; the colour of the iodine is not completely discharged.

Sulphate –To 5 ml add 10 mg of sodium bicarbonate and evaporate to dryness on a water bath; the residue, dissolved in *water*; complies with the *limit test for sulphates*, Appendix. 2.3.7.

Free chlorine –Dilute 5 ml with 10 ml of freshly boiled and cooled *water*, add 1 ml of cadmium *iodide solution*, and shake with 1 ml of *chloroform*; the chloroform layer does not become violet within one minute.

Sulphated ash –Not more than 0.01 per cent, Appendix 2.3.6.

Assay –Weigh accurately about 4 g into a stoppered flask containing 40 ml of water, and titrare with *N sodium hydroxide*, using *methyl orange solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.03646 g of HCl.

Storage –Store in glass-stoppered containers at a temperature not exceeding 30º.

Hydrochloric Acid, x N –Solution of any normality x N may be prepared by diluting 84 x ml of *hydrochloric acid* to 1000 ml with *water*.

Hydrochloric Acid –(1 per cent w/v)
Dilute 1 g of *hydrochloric acid* to 100 ml with *water.*

Dilute Hydrochloric Acid –

Description –Colourless liquid.
Arsenic, heavy metals bromoide and iodide, sulphate, free chlorine –Complies with the tests described under Hydrochloric Acid, when three times the quantity is taken for each test.

Assay –Weigh accurately about 10 g and carry out the Assay described under Hydrochloric Acid.

Storage –Store in stoppered containers of glass or other inert material, at temperature below 30º.

Hydrochloric Acid, N – HCl = 36.460

36.46 g in 1000 ml

Dilute 85 ml of hydrochloric acid with water to 1000 ml and standardise the solution as follows :

Weigh accurately about 1.5 g of anhydrous sodium carbonate, previously heated at about 270º for one hour. Dissolve it in 100 ml of *water* and add two drops of *methyl red solution*. Add the acid slowly from a burette with constant stirring, until the solution becomes faintly pink. Heat again to boiling and titrate further as necessary until the faint pink colour no longer affected by continued boiling. Each 0.5299 g of *anhydrous* sodium carbonate is equivalent to 1 ml of N hydrochloric acid.

Hydrochloric Acid, Iron-Free –Hydrochloric acid which complies with the following additonal test. Evaporate 5 ml on a water-bath nearly to dryness, add 40 ml of water, 2 ml of a 20 per cent w/v solution of citric acid and two drops of thioglycollic acid, mix, make alkaline with *dilute ammonia solution*, and dilute to 50 ml with water; no pink colour is produced.

Hydrogen Peroxide Solution – (20 Vol.) H_2O_2 = 34.02

Analytical reagent grade of commerce or *hydrogen peroxide solution* (100 Vol.) diluted with 4 volumes of water.

A colourless liquid containing about 6 per cent w/v of H_2O_2; weight per ml, about 1.02 g.

Hydrogen Sulphide – H_2S =34.08

Use laboratory cylinder grade, or prepare the gas by action of hydrochloric acid, diluted with an equal volume of *water*, on iron sulphide, the resulting gas is washed by passing it through water.

A colourless, poisonous gas, with a characteristic unpleasant odour.

Hydrogen Sulphide Solution –A recently prepared, saturated solution of hydrogen sulphide in water at 20º.

Hydrogen Sulphide solution contains about 0.45 per cent w/v of H_2S.

Hydroxylamine Hydrochloride; Hydroxylammonium Chloride – NH_2OH, HCl = 69.49

Contains not less than 97.0 per cent w/w of NH_2OH, HCI

Description –Colourless crystals, or a white, crystalline powder.

Solubility –Very soluble in water; soluble in alcohol.

Free acid –Dissolve 1.0 g in 50 ml of *alcohol*, add 3 drops of *dimethyl yellow solution* and titrate to the full yellow colour with *N sodium hydroxide*; not more than 0.5 ml of *N sodium hydroxide* is required.

Sulphated ash –Not more than 0.2 per cent, Apendix 2.3.6.

Assay –Weigh accurately about 0.1 g and dissolve in 20 ml of water, add 5 g of ferric ammonium sulphate dissolve in 20 ml of water, and 15 ml of *dilute sulphuric acid*, boil for five minutes, dilute with 200 ml of water, and titrate with 0.1 *N potassium permanganate*. Each ml of 0.1 *N potassium permanganate* is equivalent to 0.003475 g of NH_2OH, HCl.

Hydroxylamine Hydrochloride Solution –Dissolve 1 g of *hydroxylamine hydrochloride* in 50 ml of *water* and add 50 ml of *alcohol,* 1 ml of *bromophenol blue solution* and 0.1 *N sodium hydroxide* until the solution becomes green.

***Indigo Carmine** – $C_{16}H_8N_2Na_2O_8S_2$ =466.4

Analytical reagent grade.

A deep blue powder, or blue granules with a coppery lustre.

Indigo Carmine Solution –To a mixture of 10 ml of *hydrochloric acid* and 990 ml of a 20 per cent w/v solution of sulphuric acid in water, add sufficient indigo carmine to produce a solution which complies with the following test.

Add 10 ml to a solution of 1.0 mg of potassium nitrate in 10 ml of *water*, add, rapidly, 20 ml of sulphuric acid and heat to boiling; the blue colour is just discharged in one minute.

*Indian ink –General purpose grade.

Iodine – I2 = 253.8

Description – Heavy, bluish-black, brittle, rhombic prisms or plates with a metallic lustre; odour characteristic; volatile at ordinary temperatures.

Solubility – Very slightly soluble in *water*; soluble in *alcohol,* freely soluble in carbon disulphide and in *chloroform,* in *solvent ether*, *in carbon tetrachloride* and in concentrated aqueous solutions of iodides.

Chloride and Bromide –Triturate 3.5 g thoroughly with 35 ml of *water*, filter and decolorise the filtrate by the addition of a little *zinc powder*. To 25 ml of the filtrate so obtained, add 5 ml of d*ilute ammonia solution,* and then 5 ml of *silver nitrate solution* added gradually, filter; dilute the filtrate to 50 ml, and acidify gradually with 4 ml of nitric acid; the opalescence in the *limit test* for chloride, Appendix 2.3.1.

Cyanides – To 5 ml of the filtrate obtained in the test for *chloride* and *bromide* add a few drops of *ferrous sulphate solution* and 1 ml of *sodium hydroxide solution*, warm gently and acidify with hydrochloric acid, no blue or green colour is produced.

Non-volatile matter –Leaves not more than 0.1 per cent as residue when volatilised on a water-bath.

Assay –Weigh accurately about 0.5 g and dissolve in a solution of 1 g of *potassium iodide* in 5 ml of *water*. Dilute to 250 ml with *water*, add 1 ml of *dilute acetic acid*, and *titrate* with 0.1 *N sodium thiosulphate, using starch solution* as indicator. Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.01269 g of I.

Storage –Store in glass-stoppered bottles or in glass or earthen-ware containers with well waxed bungs.

Iodine, 0.1N – I = 126.90; 12.69 g in 1000 ml.

Dissolve about 14 g of *iodine* in a solution of 36 g of *potassium iodide* in 100 ml of water, add three drops of *hydrochloric* acid, dilute with water to 100 ml and standardise the solution as follows :

Weigh accurately about 0.15 g of *arsenic trioxide*, previously dried at 105º for one hour, and dissolve in 20 ml of *N Sodium hydroxide* by warming, if necessary. Dilute with 40 ml of water, add two drops of *methyl orange solution* and follow with *dilute hydrochloric acid* until the yellow colour is changed to pink. Then add 2 g of *sodium bicarbonate*, dilute with 50 ml of water, and add 3 ml of starch solution, slowly add the iodine solution from a burette until a permanent blue colour is produced. Each 0.004946 g of arsenic trioxide is equivalent to 1 ml of 0.1*N iodine*.

Iodine Solution. –Dissolve 2.0 g of iodine and 3 g of *potassium iodide* in water to produce 100 ml.

Kieselguhr –A natural diatomaceous earth, purified by heating with dilute hydrochloric acid, washing with water and drying.

Lactic Acid –$CH_3CH(OH).COOH$ = 90.08

Analytical reagent grade of commerce

Lactophenol –Dissolve 20 g of *phenol* in a mixture of 20 g of *lactic acid*, 40 g of *glycerol*, and 20 ml of water.

Lead Acetate –Sugar of lead; $(CH_3CO_2)_2$ Pb, $3H_2O$ = 379.33

Contains not less than 99.5 per cent and not more than the equivalent of 104.5 per cent of C4H6O4Pb, $3H_2O$.

Description –Small, white, transparent, monoclinic prisms, or heavy, crystalline masses; odour, acetous, taste, sweet and astringent. Efflorescent in warm air. Becomes basic when heated.

Solubility –Freely soluble in *water*, and in *glycerin*; sparingly soluble in *alcohol*.

Water-insoluble matter –Dissolve 1 g in 10 ml of recently boiled and cooled *water; a* solution is produced which is, at most, faintly opalescent and becomes clear on the addition of one drop of *acetic acid*.

Chloride –1 g complies with the *limit test* for chlorides, Appendix 2.3.1.

Copper, iron, silver, and zinc – Dissolve 0.5 g in 10 ml of *water*, add 2 ml of dilute *sulphuric acid*, allow to stand for thirty minutes, and filter; to the filtrate add an excess of *potassium ferrocyanide solution;* no precipitate or colour is produced.

Assay –Weigh accurately about 0.8 g and dissolve in a mixture of 100 ml of *water* and 2 ml of *acetic acid*, add 5 g of hexamine, titrate with 0.05 *M disodium ethylenediaminetetraacetate*, using 0*.2 ml of xylenol orange solution* as indicator, until the solution becomes pale bright yellow. Each ml of 0.05 *M disodium ethylenediaminetetraacetate* is equivelent to 0.01897 g of $C_4H_6O_4Pb$, $3H_2O$.

Storage –Preserve Lead Acetate in a well-closed container.

Lead Acetate Solution –A 10.0 per cent w/v solution *of lead acetate in carbon dioxide-free water*.

Lead Nitrate – $Pb(NO_3)_2$ = 331.21

Contains not less than 99.0 per cent of $Pb(NO_3)_2$

Description –Colourless or white crystals, or a white crystalline powder.

Solubility –Soluble in water, forming a clear, colourless solution.

Assay –Weigh accurately about 0.3 g and dissolve in 150 ml of water. Add 5 ml of dilute *acetic acid*, heat to boiling, add a slight excess of *potassium chromate solution*, and boil gently until the precipitate becomes granular; collect the precipitate in a Gooch crucible, wash it with hot water, and dry to constant weight at 120°. Each g of residue is equivalent to 1.025 g of $Pb(NO_3)_2$.

Lead Solution, Standard –See limit test for heavy metals, Appendix 2.3.3.

Liquid Paraffin –General reagent grade.

Liquid paraffin is a mixture of liquid hydrocarbons obtained from petroleum.

A transparent, colourless, oily liquid, free or nearly free from fluorescence by day light; odourless and tasteless when cold, and develops not more than a faint odour of petroleum when heated.

Solubility –Practically insoluble in water, and in alcohol; soluble in chloroform, in solvent ether and in volatile oils.

Wt. per ml. –At 25°, 0.860 to 0.904 g.

Litmus –Fragments of blue pigment prepared from various species of *Rocella lecanora* or other *lichens*. It has a characteristic odour.

Partly soluble in water and in alcohol. Gives a red colour with acids and a blue colour with alkalies (pH range, 5.0 to 8.0).

Litmus Solution –Boil 25 g of coarsely powdered litmus with 100 ml of *alcohol (90 per cent)* under a reflux condenser for one hour, and pour away the clear liquid; repeat this operation using two successive quantities, each of 75 ml, of *alcohol (90 per cent)*. Digest the extracted litmus with 250 ml of water.

Litmus Paper, Blue –Boil 10 parts of coaresely powdered litmus under reflux for one hour with 100 parts of alcohol, decant the alcohol and discard. Add to the residue a mixture of 45 parts of alcohol and 55 parts of water. After two days decant the clear liquid. Impregnate the strips of filter paper with the extract and allow to dry the paper; complies with the following test –

Sensitivity–Immerse a strip measuring 10 mm x 60 mm in 100 ml of a mixture of 10 ml of 0.02 *N hydrochloric acid* and 90 ml of *water*. On shaking the paper turns red within forty five seconds.

Litmus Paper, Red – To the extract obtained in the preparation of blue litmus paper add 2 *N hydrochloric acid* drop-wise until the blue colour becomes red. Impregnate strips of filter paper with the solution and allow to dry. The paper complies with the following test :

Sensitivity–Immerse a strip measuring 10 mm x 60 mm in 100 ml of 0.002 *N sodium hydroxide*. On shaking the paper turns blue within forty-five minutes.

Magenta Basic – Fuchsin; Rosaniline hydro-chloride; $[(H_2N.\ C_6H_4)_2C : C_6H_3(CH_3) : NH_2+]Cl$ – = 337.85.

The hydrochloride of rosaniline of such a purity that when used in the preparation of decolourised solution of magenta, a nearly colourless solution is obtained.

Description –Dark red powder, or green crystals with a metallic lustre.

Solubility –Soluble in water, giving a deep reddish-purple solution.

Sulphated ash –Not more than 5.0 per cent, Appendix 2.3.6.

Magenta Solution, Decolorised –Dissolve 1 g of basic *magenta* in 600 ml of water and cool in an ice bath; add 20 g of *sodium sulphite* dissolved in 100 ml of water; cool in an ice-bath and add, slowly with constant stirring, 10 ml of hydrochloric acid; dilute with water to 1000 ml.

If the resulting solution is turbid, it should be filtered and if brown in colour, it should be shaken with sufficient decolourising charcoal (0.2 to 0.3 g) to render it colourless and then filtered immediately. Occasionally it is necessary to add 2 to 3 ml *of hydrochloric acid*, followed by shaking, to remove the little residual pink colour. The solution resulting from any of the foregoing modifications should be allowed to stand over-night before use.

Decolourised magenta solution should be protected from light.

Magnesium Carbonate –Light hydrated basic grade of commerce, containing 42 to 45 per cent of MgO and complying with the following test :

Ammonia –Dissolve 0.50 g in 4 ml of 2 M hydrochloric acid, boil to remove carbon dioxide, and dilute with *water* to 95 ml. Add 5 ml of 5 *M sodium hydroxide* and allow to stand for one hour. Dilute 40 ml of the clear liquid to 50 ml with water and add 2 ml of *alkaline potassium-mercuric iodide solution*. Any yellow colour produced is not deeper than that produced by adding 2 ml of *alkaline potassium mercuric iodide*

solution to a mixture of 44 ml of water, 2 ml of *ammonium chloride solution*, 2 ml of 2 *M hydrochloric acid* and 2 ml of 5 *M sodium hydroxide*.

Magnesium Sulphate – $MgSO_4$, $7H_2O$ = 246.47

Description –Colourless, crystals, usually needle-like; odourless, taste, cool, saline and bitter. Effloresces in warm dry air.

Solubility –Freely soluble in water; sparingly soluble in alcohol. Dissolves slowly in glycerin.

Acidity or alkalinity – 1 g dissolved in 10 ml of water is neutral to *litmus solution*.

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.
Iron –2 g dissolved in 20 ml of water complies with the limit test for iron, Appendix 2.3.4.
Heavy metals –Not more than 10 parts per million, determined by Method A on a solution prepared by dissolving 2.0 g in 10 ml of water, 2.0 ml of *of dilute acetic acid* and sufficient *water* to make 25 ml, Appendix 2.3.3.

Zinc –Dissolve 2 g in 20 ml of water and acidify with 1 ml of *acetic acid*. No turbidity is produced immediately on the addition of few drops of *potassium ferrocyanide solution*.

Chloride –1 g complies with the *limit test for chlorides*, Appendix 2.3.2.

Loss on ignition –Between 48.0 per cent and 52.0 per cent, determined on 1.0 g by drying in an oven at 105º for two hours and igniting to constant weight at 400º.

Assay –Weigh accurately about 0.3 g and dissolve in 50 ml of *water*. Add 10 ml of *strong ammonia-ammonium chloride solution*, and titrate with 0.05 *M disodium ethylenediaminetetraacetate* using 0.1 g of *mordant black* II mixture as indicator, until the pink colour is discharged from the blue. Each ml of 0.05 *M disodium ethylenediaminetetraacetate* is equivalent to 0.00602 g of $MgSO_4$.

Storage –Store in well-closed containers.

Magnesium Sulphate, Dried, – $MgSO_4$

Dried, general reagent grade of commerce.

Magnesium Sulphate Solution, Ammoniacal –Dissolve 10 g of *magnesium sulphate* and 20 g of *ammonium chloride* in 80 ml of *water*, and add 42 ml of *5 M ammonia*. Allow to stand for a few days in a well closed container; decant and filter.

Mercuric Chloride –$HgCl_2$ =271.50.

Contains not less than 99.5 per cent of HgCl2;

Description –Heavy, colourless or white, crystalline masses, or a white crystalline powder .

Solubility –Soluble in *water*; freely soluble in *alcohol*.

Non-volatile matter –When volatilised, leaves not more than 0.1 per cent of residue.

Assay –Weigh accurately about 0.3 g and dissolve in 85 ml of *water* in a stoppered-flask, add 10 ml of *calcium chloride solution*, 10 ml of *potassium iodide solution*, 3 ml of *formaldehyde solution and* 15 ml of *sodium hydroxide solution*, and shake continuously for two minutes. Add 20 ml of acetic acid and 35 ml of 0.1 *N iodine.* Shake continuously for about ten minutes, or until the precipitated mercury is completly

redissolved, and titrate the excess of iodine with 0.1 *N sodium thiosulphate*. Each ml of 0.1 *N iodine* is equivalent to 0.01357 g of $HgCl_2$.

Mercuric Chloride, 0.2 M –

Dissolve 54.30 g of *mercuric chloride* in sufficient water to produce 1000 ml.

Mercuric Chloride Solution –A 5.0 per cent w/v solution of *mercuric chloride* in water.

Mercuric Oxide, Yellow – HgO = 216.59.

Contains not less than 99.0 per cent of HgO, calculated with reference to the substance dried at 105º for one hour.

Description – Orange-yellow, heavy, amorphous powder; odourless, stable in air but becomes discoloured on exposure to light.

Solubility –Practically insoluble in water and in *alcohol*; freely soluble in *dilute hydrochloric acid* and in dilute *nitric acid*, forming colourless solutions.

Acidity or alkalinity –Shake 1 g with 5 ml of *water* and allow to settle; the supernatant liquid is neutral to *litmus solution*.

Mercurous salts –A solution of 0.5 g in 25 ml of *dilute hydrochloric acid* is not more than slightly turbid.

Chloride – To 0.2 g add 1 g of zinc powder and 10 ml of *water*. Shake occasionally during ten minutes and filter; the solution complies with the *limit test* for chlorides, Appendix 2.3.2.

Sulphated ash –When moistened with *sulphuric acid* in a silica dish and heated strongly to constant weight, leaves not more than 0.5 per cent of residue.

Assay –Weigh accurately about 0.4 g, dissolve in 5 ml of nitric acid and 10 ml of *water* and *dilute* with *water* to 150 ml. Titrate with 0.1 *N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Carry out the titration at a temperature not above 20º. Each ml of 0*.1 N ammonium thiocyanate* is equivalent to 0.01083 g of HgO.

Storage –Preserve Yellow Mercuric Oxide in a well-closed container, protected from light.

Mercuric Potassium Iodide Solution –

See Potassium-Mercuric Iodide solution.

Mercuric Sulphate –Mercury (II) Sulphate $HgSO_4$= 296.68

Contains not less than 99.0 per cent of $HgSO_4$

Description- A white; crystalline powder, hydrolyses in water.

Solubility – Soluble in *dilute sulphuric acid*.

Chloride –Dissolve 2.0 g in a mixture of 2 ml of *dilute sulphuric acid* and 10 ml of *water*. Add 2 g of zinc powder, shake frequently for five minutes and filter. The filtrate complies with the *limit test* for *chlorides*, Appendix 2.3.2.

Nitrate –Dissolve 0.40 g in a mixture of 9 ml of water and 1 ml of dilute sulphuric acid, add 1 ml of indigo carmine solution and 10 ml of *nitrogen-free sulphuric acid* and heat to boiling, the blue colour is not entirely discharged.

Assay –Dissolve 0.6 g in a mixture of 10 ml of *dilute nitric acid* and 40 ml of *water*. Titrate with 0.1 *N ammonium thiocyanate*, using *ferric ammonium sulphate solution* as indicator. Each ml of 0.1 *N ammonium thiocyanate* is equivalent to 0.01483 g of $HgSO_4$.

Mercury Sulphate Solution – Mix 5 g of *yellow mercuric oxide* with 40 ml of *water*, and while stirring add 20 ml of *sulphuric acid*, and 40 ml of *water*, and stir until completely dissolved.

Methyl Alcohol : Methanol : CH_3OH = 32.04.

Description –Clear, Colourless liquid with a characteristic odour.

Solubility –Miscible with water, forming a clear colourless liquid.

Specific Gravity – At 25º, not more than 0.791.

Distillation range – Not less than 95 per cent distils between 64.5º and 65.5º.

Refractive Index –At 20º, 1.328 to 1.329.

Acetcone –Place 1 ml in a *Nessler cylinder*, add 19 ml of water, 2 ml of a 1 per cent w/v solution of *2-nitrobenzaldehyde* in *alcohol (50 per cent)*, 1 ml of 30 per cent w/v *solution of sodium hydroxide* and allow to stand in the dark for fifteen minutes. The colour developed does not exceed that produced by mixing 1 ml of standard acetone solution, 19 ml of water, 2 ml of the solution of *2-nitrobenzaldehyde* and 1 ml of the *solution of sodium hydroxide* and allowing to stand in the dark for fifteen minutes.

Acidity –To 5 ml add 5 ml of *carbon dioxide-free water*, and titrate with 0.1 *N sodium hydroxide*, using *bromothymol blue solution* as indicator; not more than 0.1 ml is required.

Non-volatile matter – When evaporated on a water-bath and dried to constant weight at 105º, leaves not more than 0.005 per ceant w/v of residue.

Methyl Alcohol, Dehydrated –Methyl alcohol which complies with the following additional requirement.

Water –Not more than 0.1 per cent w/w.

Methylene Blue –$C_{16}H_{18}ClN_3S, 3H_2O$. Tetramethylthionine chloride.

A dark green or bronze crystalline powder, freely soluble in water, soluble in alcohol.

Loss on drying –Not less than 18 per cent and not more than 22 per cent, determined by drying in an oven at 100º to 105º.

Methylene Blue Solution – Dissolve 0.18 g of *methylene blue* in 100 ml of *water*. To 75 ml of this solution, add 5 ml of 0.1 *N sodium hydroxide* and 20 ml of water.

Methyl Orange –Sodium-p-dimethylamineazobenzene sulphate, $C_{14}H_{14}O_3N_3SNa$.

An orange-yellow powder or crystalline scales, slightly soluble in cold water; insoluble in alcohol; readily soluble in hot water.

Methyl Orange Solution –Dissolve 0.1 g of methyl orange in 80 ml of water and dilute to 100 ml with alcohol.

Test for sensitivity –A mixture of 0.1 ml of the methyl orange solution and 100 ml freshly boiled and cooled water is yellow. Not more than 0.1 ml of 0.1 N hydrochloric acid is required to change the colour to red.

Colour change – pH 3.0 (red) to pH 4.4 (yellow).

Methyl Red –p-Dimethylaminoazobenzene-o-carboxylic acid, $C_{15}H_{15}O_2N_3$.

A dark red powder or violet crystals, sparingly soluble in *water*; soluble in alcohol.

Methyl red solution –Dissolve 100 mg in 1.86 ml of 0.1 *N sodium hydroxide* and 50 ml of *alcohol* and dilute to 100 ml with water.

Test for sensitivity –A mixture of 0.1 ml of the *methyl red solution* and 100 ml of freshly boiled and cooled *water* to which 0.05 ml of 0.02 *N hydrochloric acid* has been added is red. Not more than 0.01 ml of 0.02 *N sodium hydroxide* is required to change the colour to yellow.

Colour change – pH 4.4 (red) to pH 6.0 (yellow).

Molish's Reagent –Prepare two solutions in separate bottles, with ground glass stoppers :

(a) Dissolve 2 g of α–naphthol in 95 per cent alcohol and make upto 10 ml with alcohol (α–naphthol can be replaced by thymol or resorcinol). Store in a place protected from light. The solution can be used for only a short period.

(b) Concentrated sulphuric acid.

Mordant Black II –See Eriochrome black T.

Mordant Black II Mixture –*Mordant black mixture.*

A mixture of 0.2 part of Mordant Black II with 100 parts of sodium chloride. Mordant Black II Mixture should be recently prepared.

α–Naphthol – 1-Naphthol; $C_{10}H_7OH$=144.17.

Description – Colourless or white crystals or a white, crystalline powder; odour, characteristic.

Solubility –Freely soluble in alcohol yielding not more than slightly opalescent, colourless or almost colourless solution, with no pink tint.

Melting range –93° to 96°.

Sulphated ash –Not more than 0.05 per cent, Appendix 2.3.6.

α–Naphthol Solution – 1-Naphthol solution.

Dissolve 1 g of *α–naphthol* in a solution of 6 g of *sodium hydroxide* and 16 g of *anhydrous sodium carbonate* in 100 ml of water.

α–naphthol solution must be prepared immediately before use.

1-Naphthylamine –$C_{10}H_9N$ = 143.2 – Analytical reagent grade.

Almost colourless crystals, or a white crystalline powder; melting point, about 50º.

Naphthylamine-Sulphanilic Acid Reagent –Immediately before use mix equal volumes of solutions A and B prepared as follows :

Solution A –Dissolve 0.5 g of sulphuric acid in 30 ml of 6 M *acetic acid* and dilute to 150 ml with water.

Solution B –Dissolve 0.15 g of 1 naphthylamine in 30 ml of 6 M *acetic acid* and dilute to 150 ml with water.

Ninhydrin Reagent – 30 mg ninhydrin is dissolved in 10 ml n-butanol, followed by 0.3 ml of 98 % acetic acid.

Nitric Acid –Contains 70.0 per cent w/w of HNO3 (limits, 69.0 to 71.0). About 16 N in strength.

Description –Clear, colourless, fuming liquid.

Wt. per ml. – At 20º, 1.41 to 1.42 g.

Copper and Zinc –Dilute 1 ml with 20 ml of water, and add a slight excess of dilute ammonia solution; the mixture does not become blue. Pass hydrogen sulphide; a precipitate is not produced.

Iron –0.5 ml of complies with the limit test for iron, Appendix 2.3.4.

Lead –Not more than 2 parts per million, Appendix 2.3.5.

Chloride –5 ml neutralised with dilute ammonia solution, complies with the limit test for chlorides, Appendix 2.3.2.

Sulphates –To 2.5 ml add 10 mg of sodium bicarbonate and evaporate to dryness on a water-bath, the residue dissolved in water, complies with the limit test for sulphates, Appendix 2.3.7.

Sulphated ash –Not more than 0.01 per cent w/w, Appendix 2.3.6.

Assay –Weigh accurately about 4 g into a stoppered flask containing 40 ml of water, and titrate with N Sodium hydroxide, using methyl orange solution as indicator. Each ml of N sodium hydroxide is equivalent to 0.06301 g of HNO_3.

Nitric Acid, XN –Solutions of any normality XN may be prepared by diluting 63x ml of nitric acid to 1000 ml with water.

Nitric Acid, Dilute –Contains approximately 10 per cent w/w of HNO_3. Dilute 106 ml of nitric acid to 1000 ml with water.

2-Nitrobenzaldehyde –0-Nitrobenzaldehyde $NO_2C_6H_4CHO$ =151.12.

Description –Yellow needles, odour, resembling that of benzaldehyde.

Solubility –Soluble in alcohol.

Melting range –40º to 45º.

Sulphated ash – Not more than 0.1 per cent, Appendix 2.3.6.

Oxalic Acid –$(CO_2H)_2$, $2H_2O$ =126.07.

Contains not less than 99.0 per cent of $C_2H_2O_4$, $2H_2O$, as determined by the methods A and B under the Assay.

Description –Colourless crystals.

Solubility – Soluble in water and in alcohol.

Chloride – To 1 g dissolved in 20 ml of water add 5 ml. of dilute *nitric acid* and 1 drop of silver nitrate solution; no turbidity is produced.

Sulphated ash –Not more than 0.05 per cent, Appendix 2.3.6.

Assay –

- Weigh accurately about 3 g and dissolve in 50 ml of carbon dioxide free water and titrate with N sodium hydroxide, using phenolphtahalein solution as indicator. Each ml of N sodium hydroxide is equivalent to 0.06304 of $C_2H_2O_4$, $2H_2O$.

- Weigh accurately about 3 g, dissolve in water, and add sufficient water to produce 250 ml. To 25 ml of this solution add 5ml of sulphuric acid previously diluted with a little water, and titrate at a temperature of about 70º with 0.1N potassium permanganate. Each ml of 0.1 *N potassium permanganate* is equivalent to 0.006303 g of $C_2H_2O_4$, $2H_2O$.

Oxalic Acid, 0.1 N – $C_2H_2O_4$, $2H_2O$ = 126.07, 6.303 g in 1000 ml.

Dissolve 6.45 g of oxalic acid in sufficient water to produce 1000 ml and standardise the solution as follows:

Pipette 30 ml of the solution into a beaker, add 150 ml of water, 7 ml of *sulphuric acid* and heat to about 70º. Add slowly from a burette freshly standardised 0.1 *N potassium permanganate* with constant stirring, until a pale-pink colour, which persists for fifteen seconds, is produced. The temperature at the conclusion of the titration should not be less than 60°. Each ml of 0.1 *N potassium permanganate* is equivalent to 0.006303 g of $H_2C_2O_4$, $2H_2O$.

Petroleum Light – Petroleum Spirit

Description –Colourless, very volatile, highly flammable liquid obtained from petroleum, consisting of a mixture of the lower members of the paraffin series of hydrocarbons and complying with one or other of the following definitions :

Light Petroleum –(Boiling range, 30º to 40º).

Wt. per ml. –At 20º, 0.620 to 0.630 g.

Light Petroleum –(Boiling range, 40º to 60º).

Wt. per ml –At 20º, 0.630 to 0.650 g.

Light Petroleum –(Boiling range, 60º to 80º).

Wt. per ml. –At 20º, 0.670 to 0.690.

Light Petroleum –(Boiling range, 80º to 100º).

Wt. per ml. –At 20º, 0.700 to 0.720

Light Petroleum –(Boiling range, 100º to 120º).

Wt. per ml –At 20º, 0.720 to 0.740 g.

Light Petroleum –(Boiling range, 120º to 160º).

Wt. per ml –At 20º, about 0.75 g.

Non-volatile matter –When evaporated on a water-bath and dried at 105º, leaves not more than 0.002 per cent w/v of residue.

Phenacetin – $C_{10}H_{13}O_2N$ = 179.2

Analytical reagent grade.

White, glistening, crystalline scales, or a fine, white, crystalline powder; odourless; taste, slightly bitter.

Melting range –134º to 136º.

Phenol – C_6H_5OH = 94.11

Analytical reagent grade.

Caustic, deliquescent crystals with a characteristic odour; freezing point, about 41º.

Phenol Liquified –General reagent grade.

A solution in water containing about 80 per cent w/w C_6H_6O.

Phenol Red –$C_{19}H_{14}O_5S$. Phenolsulphonphthalein.

A light to dark red crystalline powder, very slightly soluble in water, slightly soluble in alcohol, soluble in dilute alkaline solutions.

Phenol Red Solution –Dissolve 0.10 g of *phenol red* in 2.82 ml of 0.1 *N sodium hydroxide*, and add 20 ml of *alcohol* and dilute to 100 ml with water.

Test for sensitivity –A mixture of 0.1 ml of the *phenol red solution* in 100 ml of freshly boiled and cooled water is yellow. Not more than 0.1 of 0.02 *N* sodium hydroxide is required to change the colour to red-violet.

Colour change - pH 6.8 (yellow) to pH 8.4 (red-violet).

Phenolphthalein –$C_{20}H_{14}O_4$.

A white to yellowish-white powder, practically insoluble in water, soluble in alcohol.

Phenolphthalein Solution –Dissolve 0.10 g in 80 ml of *alcohol* and dilute to 100 ml with water.

Test for sensitivity –To 0.1 ml of the *phenolphthalein solution* add 100 ml of freshly boiled and cooled water, the solution is colourless. Not more than 0.2 ml of 0.02 *N sodium hydroxide* is required to change the colour to pink.

Colour change –pH 8.2 (colourless) to pH 10.0 (red)

Phloroglucinol – 1 : 3 : 5 – Trihydroxybenzene , $C_6H_3(OH)_3$, $2H_2O$.

Description – White or yellowish crystals or a crystalline powder.

Solubility –Slightly soluble in water; soluble in *alcohol*, and in *solvent ether*.

Melting range –After drying at 110º for one hour, 215º to 219º.

Sulphated ash – Not more than 0.1 per cent, Appendix 2.3.6.

Phloroglucinol should be kept protected from light.

Phloroglucinol Solution –A 1.0 per cent w/v solution of phloroglucinol in alcohol (90 per cent).

Phosphoric Acid – H_3PO_4 = 98.00.

(Orthophosphoric Acid; Concentrated Phosphoric Acid).

Description –Clear and colourless syrupy liquid, corrosive.

Solubility –Miscible with water and with alcohol.

Hypophoshorous and phosphorous acid – To 0.5 ml add 10 ml of water and 2 ml of *silver nitrate solution* and heat on a waterbath for five minutes; the solution shows no change in appearance.

Alkali phosphates - To 1 ml in a graduated cylinder add 6 ml of *solvent ether* and 2 ml of *alcohol*; no turbidity is produced.

Chloride –1 ml complies with the limit test for chlorides, Appendix 2.3.2.

Sulphate –0.5 ml complies with the limit test for sulphate, Appendix 2.3.7.

Arsenic – Not more than 2 parts per million, Appendix 2.3.1.

Heavy metals –Not more than 10 parts per million, determined by Method A on a solution prepared by diluting 1.2 ml with 10 ml of *water*, neutralising with dilute *ammonia solution*, adding sufficient dilute *acetic acid* to render the solution acidic and finally diluting to 25 ml with *water*, Appendix 2.3.3.

Iron –0.1 ml complies with the limit test for iron, Appendix 2.3.4.

Aluminium and calcium –To 1 ml add 10 ml of water and 8 ml of dilute *ammonia solution* the solution remains clear.

Assay –Weigh accurately about 1 g. and mix with a solution of 10 g of *sodium chloride* in 30 ml of water. Titrate with *N sodium hydroxide*, using *phenolphthalein solution* as indicator. Each ml of *N sodium hydroxide* is equivalent to 0.049 g of H_3O_4

Storage –Store in a well-closed glass containers.

Phosphoric Acid, xN –

Solutions of any normality, x N may be prepared by diluting 49 x g of *phosphoric acid* with water to 1000 ml.

Phosphoric Acid, Dilute –

Contains approximately 10 per cent w/v of H_3O_4.

Dilute 69 ml of *phosphoric acid* to 1000 ml with water.

Piperazine Hydrate –$C_4H_{10}N_2$, $6H_2O$ = 194.2.

General reagent grade of commerce.

Colourless, glossy, deliquescent crystals, melting point, about 44º.

Potassium Antimonate –$KSbO_3$, $3H_2O$ =262.90.

Contains not less than 40.0 per cent of Sb.

Description – White, crystalline powder.

Solubility –Sparingly soluble in water, very slowly soluble in cold, but rapidly soluble on boiling.

Assay –Weigh accurately about 0.3 g, and dissolve in 100 ml of water, add 2 ml of dilute *hydrochloric acid*, and pass in *hydrogen sulphide* until the *antimony* is completely precipitated. Add 2 ml of *hydrochloric acid* and again pass in *hydrogen sulphide.* Boil, filter, wash the precipitate with hot *water* saturated with *hydrogen sulphide,* and dissolve the precipitate in 25 ml of *hydrochloric acid*. Boil to remove *hydrogen sulphide*, and dilute to 50 ml with *water.* Add 2 g of *sodium potassium tartrate,* neutralise carefully with *sodium carbonate,* add 2 g *sodium bicarbonate,* and titrate with 0.1 *N iodine, using starch solution* as indicator. Each ml of 0.1 *N iodine* is equivalent to 0.006088 g of Sb.

Potassium Antimonate Solution –Boil 2 g of *potassium antimonate* with 95 ml of *water* until dissolved. Cool rapidly and add 50 ml of *potassium hydroxide solution* and 5 ml of *N sodium hydroxide.* Allow to stand twenty-four hours, filter and and sufficient water to produce 150 ml.

Sensitivity to sodium –To 10 ml add 7 ml of 0.1 M *sodium chloride,* a white crystalline precipitate is formed within fifteen minutes.

Potassium Antimonate Solution should be freshly prepared.

Potassium Bisulphate – Potassium Hydrogen Sulphate; $KHSO_4$ = 136.16.

Contains not less than 98.0 per cent and not more than the equivalent of 102.0 per cent of $KHSO_4$.

Description – Fused, white lumps; hygroscopic.

Solubility –Very soluble in water, giving an acid solution.

Iron–2 g complies with the limit test for iron, Appendix 2.3.4.

Assay– Weigh accurately about 4.5 g, dissolve in 50 ml of *water* and titrate with *N sodium hydroxide* using *methyl red solution as* indicator. Each ml of *N sodium hydroxide* is equivalent to 0.1362 g of $KHSO_4$

Potassium Bromate – $KBrO_3$ = 167.00

Contains not less than 99.8 per cent of $KBrO_3$ calculated with reference to the substance dried to constant weight at 105°.

Description –White, crystalline powder.

Solubility – Soluble in *water,* freely soluble in boiling water, almost insoluble in *alcohol.*

Acidity or Alkalinity – A 5 per cent w/v solution in *water* is clear and colourless and neutral to *litmus solution.*

Sodium –A warm 10 per cent w/v solution in *water,* tested on platinum wire, imparts no distinct yellow colour to a colourless flame.

Bromide –To 20 ml of a 5 per cent w/v solution in *water* , add 1 ml of 0.1 *N sulphuric acid;* no yellow colour develops within one minute, comparison being made with a control solution to which no acid has been added.

Sulphate –1 g complies with the limit test for *sulphates,* Appendix 2.3.7.

Assay –Weigh accurately about 1 g, dissolve in water and dilute to 250 ml. To 25 ml of this solution add 3 g of *potassium iodide* and 10 ml of *hydrochloric acid,* dilute with 100 ml of water and titrate with 0.1 *N sodium thiosulphate,* using *starch solution* as indicator. Each ml of 0.1 *N sodium thiosulphate* is equivalent 0.002783 g of $KBrO_3$.

Potassium Bromide –KBr = 119.0

Analytical reagent grade.

Potassium Bromide, 0.001 N –

Dissolve 0.1190 g of *potassium bromide* in sufficient *water* to produce 1000 ml.

Potassium Carbonate –$K2CO_3$ = 138.21

Contains not less than 98.0 per cent of $K2CO_3$.

Description –White, granular powder, hygroscopic.

Solubility –Very soluble in *water,* forming a clear solution.

Iron – 1 g, with the addition of 1.5 ml of *hydrochloric acid,* complies with the limit test for *iron,* Appendix 2.3.4.

Chloride –1g, with the addition of 5 ml of *nitric acid,* complies with the limit test for *chlorides*, Appendix 2.3.2.

Sulphate –1 g, with the addition of 5 ml of *hydrochloric acid,* complies with the limit test for *sulphates,* Appendix 2.3.7.

Chromium –To 25 ml of a 2 per cent w/v solution in *water,* add about 0.2 g of *sodium peroxide and* boil gently for five minutes, cool, acidify with *dilute sulphuric acid* and add 2 drops of *diphenylcarbazide solution;* no violet colour is produced.

Assay –Weigh accurately about 3 g, dissolve in 50 ml of *water,* and titrate with *N hydrochloric acid,* using *bromophenol blue solution* as indicator. At the first colour change, boil the solution, cool, and complete the titration. Each ml of *N hydrochloric acid* is equivalent to 0.06911 g of $K2CO_3$.

Potassium Carbonate, Anhydrous. –Potassium carbonate dried at 135° for two hours spread in a thin layer and then cooled in a desiccator.

Potassium Chlorate – $KClO_3$ =122.55

Contains not less than 99.0 per cent of $KClO_3$.

Description –White powder or colourless crystals. In admixture with organic or readily oxidisable substances, it is liable to explode if heated or subjected to percussion or trituration.

Solubility –Soluble in *water*, and in *glycerin*; practically insoluble in *alcohol.*

Lead –Not more than 10 parts per million, Appendix 2.3.5.

Chloride –0.5 g complies with the limit test for *chlorides,* Appendix 2.3.2.

Sulphate –0.5 g complies with the limit test for *sulphates,* Appendix 2.3.7.

Assay –Weigh accurately about 0.3 g and dissolve in 10 ml of *water* in a stoppered-flask, add 1 g of *sodium nitrate,* dissolved in 10 ml of *water,* and then 20 ml of *nitric acid;* stopper the flask and allow to stand for ten minutes; and 100 ml of water and sufficient *potassium permangnate solution* to produce a permanent pink colour; decolorise by the addition of a trace of *ferrous sulphate* and add 0.1 g of *urea.* Add 30 ml of 0.1 *N silver nitrate,* filter, wash with *water*, and titrate the filtrate and washings with 0.1 *N ammonium thiocyanate,* using *ferric ammonium sulphate solution* as indicator. Each ml of 0.1 *N silver nitrate* is equivalent to 0.01226 g of $KClO_3$

Potassium Chloride –KCl = 74.55

Analytical reagent grade

Potassium Chromate – K_2CrO_4 = 194.2

Analytical reagent grade

Potassium Chromate Solution –A 5.0 per cent w/v solution of potassium chromate.

Gives a red precipitate with *silver nitrate* in neutral solutions.

Potassium Cupri-Tartrate Solution –Cupric Tatrate Alkaline Solution : Fehling's Solution.

A. **Copper Solution –**Dissolve 34.66 g of carefully selected small crystals of *copper sulphate,* showing no trace of efflorescence or of adhering moisture, in sufficient water to make 500 ml. Keep this solution in small, well-stoppered bottles

B. **Alkaline Tartrate Solution –** Dissolve 176 g of sodium *potassium tartrate* and 77 g of *sodium hydroxide* in sufficient *water* to produce 500 ml.

Mix equal volumes of the solutions No. 1 and No. 2 at the time of using.

Potassium Cyanide –KCN =65.12

Contains not less than 95.0 per cent of KCN.

Description –White, crystalline powder, gradually decomposing on exposure to air.

Solubility –Readily soluble in *water,* forming a clear, colourless solution.

Heavy metals – To 20 ml of a 5 per cent w/v solution in *water,* add 10 ml of *hydrogen sulphide solution;* no darkening is produced immediately or on the addition of 5 ml of *dilute hydrochloric acid.*

Assay – Weigh accurately about 0.5 g and dissolve in 50 ml of *water*, add 5 ml of dilute *ammonia solution* and 1 drop of *potassium iodide solution*; titrate with 0.1 *N silver nitrate until a faint* permanent turbidity appears. Each ml of 0.1 *N silver nitrate* is equivalent to 0.01302 g of KCN.

Potassium Cyanide Solution –A 10.0 per cent w/v solution of *potassium cyanide* in *water.*

Potassium Cyanide Solution, Lead –free –Weigh accurately about 10 g of *potassium cyanide* and dissolve in 90 ml of *water,* add 2 ml of *hydrogen peroxide solution,* allow to stand for twenty-four hours, and make up to 100 ml with *water*. It complies with the following tests.

Mix 2 ml with 5 ml of *lead-free ammonia solution* and 40 ml of water, and add 5 ml of *standard lead solution;* no darkening is produced.

Potassium Dichromate – $K_2Cr_2O_7$ =294.18.

Contains not less than 99.8 per cent of $K_2Cr_2O_7$

Description – Orange-red crystals or a crystalline powder.

Solubility – Soluble in *water*

Chloride. –To 20 ml of a 5 per cent w/v solution in *water* and 10 ml *nitric acid,* warm to about 50º and add a few drops of *silver nitrate solution*; not more than a faint opalescence is produced.

Assay –Carry out the Assay described under Potassium Chromate, using 2 g. Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.004904 g of $K_2Cr_2O_7$.

Potassium Dichromate Solution – A 7.0 per cent w/v solution of *potassium dichromate* in *water.*

Potassium Dichromate, Solution 0.1N – $K_2Cr_2O_7$ = 294.18, 4.903 g in 1000 ml.
Weigh accurately 4.903 g of *potassium dichromate* and dissolve in sufficient *water* to produce 1000 ml.

Potassium Dihydrogen Phosphate - KH_2PO_4 = 136.1

Analytical reagent grade of commerce.

Potassium Ferricyanide – $K_3Fe(CN)_6$ = 329.25

Contains not less than 99.0 per cent of $K_3Fe(CN)_6$

Description –Ruby-red crystals.

Solubility –Very soluble in *water.*

Ferrocyanide –Rapidly wash 1 g with water, then dissolve in 100 ml of *water,* and add 1 drop of *ferric ammonium sulphate solution;* no blue colour is produced.

Assay –Weigh accurately about 1 g and dissolve in 50 ml of *water,* add 5 g of *potassium iodide* and 3 g of *zinc sulphate*, and titrate the liberated *iodine* with 0.1 *N sodium thiosulphate, using starch solution*, added towards the end of the titration, as indicator.Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.03293 g of $K_3Fe(CN)_6$.

Potassium Ferricyanide Solution –Wash about 1 g of *potassium ferricyanide* crystals with a little *water,* and dissolve the washed crystals in 100 ml of *water*.

Potassium Ferricyanide solution must be freshly prepared.

Potassium Ferrocyanide – $K_4Fe(CN)_6$, $3H_2O$ =422.39

Contains not less than 99.0 per cent of $K_4Fe(CN)_6$, $3H_2O$.

Description –Yellow, crystalline powder.

Solubility –Soluble in water.

Acidity or Alkalinity –A 10 per cent w/v solution in water is neutral to litmus paper.

Assay –Weigh accurately about 1g and dissolve in 200 ml of *water,* add 10 ml of *sulphuric acid* and titrate with 0.1 *N potassium permanganate.* Each ml of 0.1 *N potassium permanganate* is equivalent to 0.04224 g of $K_4Fe(CN)_6$, $3H_2O$.

Potassium Ferrocyanide Solution –A 5.0 per cent w/v solution of *potassium ferrocyanide in water.*

Potassium Hydrogen Phthalate –CO_2H. C_6H_4. CO_2K =204.22.

Contains not less than 99.9 per cent and not more than the equivalent of 100.1 per cent of $C_8H_5O_4K$ calculated with reference to the substance dried at 110º for one hour.

Description –White, crystalline powder.

Solubility –Slowly soluble in *water,* forming clear, colourless solution.

Acidity –A 2.0 per cent w/v solution in carbon dioxide free water gives with *bromophenol blue solution* the grey colour indicative of pH 4.0.

Assay –Weigh accurately about 9 g, dissolve in 100 ml of *water* and titrate with *N sodium hydroxide* using *phenolphthalein solution* as indicator. Each ml of *N Sodium hydroxide* is equivalent to 0.2042 g of $C_8H_5O_4K$.

Potassium Hydrogen Phthalate, 0.02 M –

Dissolve 4.084 g of *Potassium hydrogen phthalate* in sufficient *water* to produce 1000 ml.

Potassium Hydrogen Phthalate, 0.2 M –

Dissolve 40.84 g of *potassium hydrogen phthalate* in sufficient *water* to produce 1000 ml.

Potassium Hydroxide –Caustic Potash : KOH = 56.11

Contains not less than 85.0 per cent of total alkali, calculated as KOH and not more than 4.0 per cent of K_2CO_3.

Description –Dry white sticks, pellets or fused mass; hard, brittle and showing a crystalline fracture; very deliquescent; strongly alkaline and corrosive.

Solubility –Freely soluble in *water*, in *alcohol* and in *glycerin;* very soluble in boiling ethyl alcohol.

Aluminium, iron and matter insoluble in hydrochloric acid –Boil 5 g with 40 ml of d*ilute hydrochloric acid,* cool, make alkaline with *dilute ammonia solution,* boil, filter and wash the residue with a 2.5 per cent w/v solution of *ammonium nitrate*; the insoluble residue, after ignition to constant weight, weighs not more than 5 mg.

Chloride –0.5 g dissolved in water with the additon of 1.6 ml of *nitric acid*, complies with the limit test for *chlorides*, Appendix 2.3.2.

Heavy metals –Dissolve 1 g in a mixture of 5 ml of *water* and 7 ml of *dilute hydrochloric acid.* Heat to boiling, add 1 drop of *phenolphthalein solution* and *dilute ammonia solution* dropwise to produce a faint pink colour. Add 2 ml of *acetic acid* and *water* to make 25 ml; the limit of heavy metals is 30 parts per million, Appendix 2.3.3.

Sulphate –Dissolve 1 g in water with the addition of 4.5 ml of *hydrochloric acid;* the solution complies with the limit test for *sulphates,* Appendix 2.3.7.

Sodium –To 3 ml of a 10 per cent w/v solution add 1 ml of *water*, 1.5 ml of *alcohol,* and 3 ml of *potassium antimonate solution* and allow to stand; no white crystalline precipitate or sediment is visible to the naked eye within fifteen minutes.

Assay –Weigh accurately about 2 g, and dissolve in 25 ml of *water,* add 5 ml of *barium chloride solution,* and titrate with *N hydrochloric acid, u*sing *phenolphthalein solution* as indicator. To the solution in the flask add *bromophenol blue solution,* and continue the titration with *N hydrochloric* acid. Each ml of *N hydrochloric acid,* used in the second titration in equivalent to 0.06911 g of K_2CO_3 . Each ml of *N hydrochloric acid,* used in the combined titration is equivalent to 0.05611 g of total alkali, calculated as KOH.

Storage –Potassium Hydroxide should be kept in a well-closed container.

Potassium Hydroxide, xN –

Solution of any normality, x N, may be prepared by dissolving 56.11x g of *potassium hydroxide* in *water* and diluting to 1000 ml.

Potassium Hydroxide Solution –Solution of Potash.

An aqueous solution of *potassium hydroxide* containing 5.0 per cent w/v of total alkali, calculated as KOH (limits, 4.75 to 5.25).

Assay –Titrate 20 ml with *N sulphuric acid,* using *solution of methyl orange* as indicator. Each ml of *N sulphuric acid* is equivalent to 0.05611 g of total alkali, calculated as KOH.

Storage –*Potassium hydroxide* solution should be kept in a well-closed container of lead-free glass or of a suitable plastic.

Potassium Iodate – KIO_3 = 214.0

Analytical reagent grade.

Potassium Iodate Solution – A 1.0 per cent w/v solution of potassium iodate in water.

Potassium Iodate, 0.05 M – KIO_3 – 214.0; 10.70 g in 1000 ml

Weigh accurately 10.700 g of *potassium iodate, previously* dried at 110º to constant weight, in sufficeint water to produce 1000 ml.

Potassium Iodide –KI = 166.00

Description –Colourless crystals or white powder; odourless, taste, saline and slightly bitter.

Solubility –Very soluble in *water* and in *glycerin;* soluble in *alcohol.*

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.

Heavy metals -Not more than 10 parts per million, determined on 2.0 g by Method A, Appendix 2.3.3.

Barium –Dissolve 0.5 g in 10 ml of *water* and add 1 ml of *dilute sulphuric acid;* no turbidity develops within one minute.

Cyanides –Dissolve 0.5 g in 5 ml of warm *water,* add one drop of *ferrous sulphate solution* and 0.5 ml of *sodium hydroxide solution* and acidify with *hydrochloric acid;* no blue colour is produced.

Iodates –Dissolve 0.5 g in 10 ml of freshly boiled and cooled *water,* and add 2 drops of *dilute sulphuric acid* and a drop of starch solution; no blue colour is produced within two minutes.

Assay –Weigh accurately about 0.5 g, dissolve in about 10 ml of *water* and add 35 ml of *hydrochloric acid* and 5 ml of *chloroform.* Titrate with 0.05 M *potassium iodate* until the purple colour of iodine disappears from the chloroform. Add the last portion of the iodate solution drop-wise and agitate vigorously and continuously. Allow to stand for five minutes. If any colour develops in the chloroform layer continue the titration. Each ml of 0.05 M *potassium iodate* is equivalent to 0.0166 mg of KI.

Storage –Store in well-closed containers.

Potassium Iodide, M –Dissolve 166.00 g of *potassium iodide* in sufficient *water* to produce 1000 ml.

Potassium Iodide and Starch Solution –Dissolve 10 g of *potassium iodide* in sufficeint water to produce 95 ml and add 5 ml of *starch solution.*

Potassium Iodide and Starch solution must be recently prepared.

Potassium Iodide Solution –A 10 per cent w/v solution of *potassium iodide* in *water.*

Potassium Iodobismuthate Solution –Dissolve 100 g of *tartaric acid* in 400 ml of *water* and 8.5 g of *bismuth oxynitrate.* Shake during one hour, add 200 ml of a 40 per cent w/v solution of potassium iodide, and shake well. Allow to stand for twenty four hours and filter.

Potassium Iodobismuthate Solution, Dilute –Dissolve 100 g of *tartaric acid* in 500 ml of *water* and add 50 ml of *potassium iodobismuthate solution.*

Potassium Mercuric-Iodide Solution –Mayer's Reagent.

Add 1.36 g of *mercuric chloride* dissolved in 60 ml of *water* to a solution of 5 g of p*otassium iodide* in 20 ml of *water,* mix and add sufficient water to produce 100 ml.

Potassium Mercuri-Iodide Solution, Alkaline (Nessler's Reagent)

To 3.5 g of *potassium iodide* add 1.25 g of *mercuric chloride* dissolved in 80 ml of *water*, add a cold saturated solution of *mercuric chloride* in *water,* with constant stirring until a slight red precipitate remains. Dissolve 12 g of *sodium hydroxide* in the solution, add a little more of the cold saturated solution of *mercuric chloride* and sufficient *water* to produce 100 ml. Allow to stand and decant the clear liquid.

Potassium Nitrate - KNO_3 = 101.1

Analytical reagent grade.

Potassium Permanganate – $KMnO_4$ = 158.03

Description –Dark purple, slender, prismatic crystals, having a metallic lustre, odourless; taste, sweet and astringent.

Solubility –Soluble in *water*; freely soluble in *boiling water.*

Chloride and Sulphate –Dissolve 1 g in 50 ml of boiling *water*, heat on a water-bath, and add gradually 4 ml or a sufficient quantity of *alcohol* until the meniscus is colour-less; filter. A 20 ml portion of the filtrate complies with the limit test for *chloride,* Appendix 2.3.2., and another 20 ml portion of the filtrate complies with the limit test for *sulphates,* Appendix 2.3.7.

Assay –Weigh accurately about 0.8 g, dissolve in water and dilute to 250 ml. Titrate with this solution 25.0 ml of 0.1 *N oxalic acid* mixed with 25 ml of *water* and 5 ml of *sulphuric acid.* Keep the temperature at about 70º throughout the entire titration. Each ml of 0.1 *N oxalic acid* is equivalent to 0.00316 g of $KMnO_4$.

Storage –Store in well-closed containers.

Caution –Great care should be observed in handling *potassium permanganate*, as dangerous explosions are liable to occur if it is brought into contact with organic or other readily oxidisable substance, either in solution or in the dry condition.

Potassium Permanganate Solution – A 1.0 per cent w/v solution of *potassium permanganate* in water.

Potassium Permanganate, 0.1 N Solution –158.03. 3.161 g in 1000 ml

Dissolve about 3.3. g of *potassium permanganate* in 1000 ml of *water*, heat on a water-bath for one hour and allow to stand for two days. Filter through glass wool and standardise the solution as follows :

To an accurately measured volume of about 25 ml of the solution in a glass stoppered flask add 2 g of *potassium iodide* followed by 10 ml of *N sulphuric acid.* Titrate the liberated *iodine* with standardised 0.1 *N sodium thiosulphate,* adding 3 ml of *starch solution* as the end point is approached. Correct for a blank run on the same quantities of the same reagents. Each ml of 0.1 *N sodium thiosulphate* is equivalent to 0.003161 g of $KMnO_4$

Potassium Tetraoxalate - $KH_3(C_2O_4)_2, 2H_2O$ = 254.2.

Analytical reagent grade of commerce.

Potassium Thiocyanate –KCNS =97.18.

Analytical reagent grade.

Purified Water –H_2O = 18.02.

Description –Clear, colourless liquid, odourless, tasteless.

Purified water is prepareed from potable water by distillation, ion-exchange treatment, reverse osmosis or any other suitable process. It contains no added substances.

***p*H –** Between 4.5 and 7.0 determined in a solution prepared by adding 0.3 ml of a saturated solution of *potassium chloride* to 100 ml of the liquid being examined.

Carbon dioxide –To 25 ml add 25 ml of *calcium hydroxide solution*, no turbidity is produced.

Chloride –To 10 ml add 1 ml of *dilute nitric acid* and 0.2 ml of *silver nitrate solution;* no opalescence is produced.

Sulphate –To 10 ml add 0.1 ml of *dilute hydrochloric acid* and 0.1 ml of *barium chloride solution :* the solution remains clear for an hour.

Nitrates and Nitrites –To 50 ml add 18 ml of *acetic acid* and 2 ml of *naphthylamine-sulphanilic acid* reagent. Add 0.12 g of *zinc reducing mixture* and shake several times. No pink colour develops within fifteen minutes.

Ammonium – To 20 ml add 1 ml of *alkaline potassium mercuric-iodide solution* and after five minutes view in a Nessler cylinder placed on a white tile; the colour is not more intense than that given on adding 1 ml of *alkaline potassium mercuric-iodide solution* to a solution containing 2.5 ml of *dilute ammonium chloride solution (Nessler's)* 7.5 ml of the liquid being examined.

Calcium –To 10 ml add 0.2 ml of *dilute ammonia solution* and 0.2 ml of *ammonium oxalate solution;* the solution remains clear for an hour.

Heavy metals –Adjust the pH of 40 ml to between 3.0 and 4.0 with *dilute acetic acid,* add 10 ml of freshly prepared *hydrogen sulphide solution* and allow to stand for ten minutes; the colour of the solution is not more than that of a mixture of 50 ml of the liquid being examined and the same amount of *dilute acetic acid* added to the sample.

Oxidisable matter –To 100 ml add 10 ml of *dilute sulphuric acid* and 0.1 ml of 0.1 *N potassium permanganate* and boil for five minutes. The solution remains faintly pink.

Total Solids –Not more than 0.001 per cent w/v determined on 100 ml by evaporating on a water bath and drying in an oven at 105º for one hour.

Storage –Store in tightly closed containers.

Resorcinol –Benzene –1,3 diol; $C_6H_4(OH)_2$ = 110.1

Analytical reagent grade.

Colourless crystals or crystalline powder, melting point about 111º.

Resorcinol Solution –

Shake 0.2 g of *resorcinol* with 100 ml of toluene until saturated and decant.

Safranine – Basic red 2

Microscopical staining grade.

A reddish-brown powder.

Safranine Solution –

Saturated solution of *safranine* in *ethanol* (70 per cent.)

Sesame Oil –

Description – A pale yellow oil, odour, slight; taste, bland.

Solubility –Slightly soluble in alcohol; miscible with *chloroform,* with *solvent ether,* with *light petroleum* (b.p. 40º to 60º) and with *carbon disulphide.*

Refaractive index – At 40º, 1.4650 to 1.4665.

Wt. Per ml – At 25º, 0.916 to 0.921 g.

Storage –Preserve sesame oil in well-closed container protected from light, and avoid exposure to excessive heat.

Silver Carbonate – Ag2 CO3 = 214

Prepared from *silver nitrate* and soluble *carbonate solution.* Light yellow powder when freshly precipitated, but becomes darker on drying and on exposure to light.

Silica Gel –

Partially dehydrated, polymerised, colloidal silicic acid containing cobalt chloride as an indicator.

Description –Blue granules, becoming pink when the moisture absorption capacity is exhausted. Silica Gel absorbs about 30 per cent of its weight of water at 20º. Its absorptive capacity may be regenerated by heating at 150º for two hours.

Silver Nitrate –$AgNO_3$ = 169.87

Description –Colourless crystals or white crystalline powder; odourless; taste, bitter and metallic.

Solubility –Very soluble in *water*, sparingly soluble in *alcohol;* slightly soluble in *solvent ether.*

Clarity and colour of solution –A solution of 2 g in 20 ml of water is clear and colourless.

Bismuth, Copper and Lead –To a solution of 1 g in 5 ml of *water, add a slight excess* of dilute *ammonia solution;* the mixute remains clear and colourless.

Foreign substances –To 30 ml of 4. 0 per cent w/v solution add 7.5 ml of 2 *N hydrochloric acid,* shake vigorously, filter and evaporate 10 ml of the filtrate to dryness on a water-bath; the residue weighs not more than 1 mg.

Assay – Weigh accurately about 0.5 g and dissolve in 50 ml of *water,* add 2 ml of *nitric acid,* and titrate with 0.1 *N ammonium thiocyanate,* using *ferric ammonium sulphate solution* as indicator. Each ml of 0.1 *N ammonium thiocyanate* is equivalent to 0.01699 g of $Ag NO_3$.

Storage –Store in tightly-closed, light resistant containers.

Silver Nitrate Solution –

A freshly prepared 5.0 per cent w/v solution of silver nitrate in water.

Silver Nitrate, 0.1 N– Ag NO_3 = 169. 87; 16.99 g in 1000 ml. Dissolve about 17 g in sufficient *water* to produce 1000 ml and standardise the solution as follows:

Weigh accurately about 0.1 g of *sodium chloride* previously dried at 110º for two hours and dissolve in 5 ml of *water*. Add 5 ml of *acetic acid*, 50 ml of *methyl alcohol* and three drops of *eosin solution is* equivalent to 1 ml of 0.1 *N silver nitrate.*

Sodium Bicarbonate – $NaHCO_3$ =84.01

Description –White, crystalline powder or small, opaque, monoclinic crystals; odourless; taste, saline.

Solubility –Freely soluble in *water*; practically insoluble in *alcohol.*

Carbonate –pH of a freshly prepared 5.0 per cent w/v solution in *carbon dioxide-free water,* not more than 8.6.

Aluminium, calcium and insoluble matter –Boil 10 g with 50 ml of *water* and 20 ml of *dilute ammonia solution,* filter, and wash the residue with water; the residue, after ignition to constant weight, not more than 1 mg.

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.

Iron –Dissolve 2.5 g in 20 ml of *water* and 4 ml of *iron-free hydrochloric acid,* and *dilute* to 40 ml with *water*; the solution complies with the *limit test for iron,* Appendix 2.3.4.

Heavy metals –Not more than 5 parts per million, determined by Method A on a solution prepared in the following manner :

Mix 4.0 g with 5 ml of *water* and 10 ml of *dilute hydrochloric acid,* heat to boiling, and maintain the temperature for one minute. Add one drop of *phenolphthalein solution* and sufficient *ammonia solution* drop wise to give the solution a faint pink colour. Cool and dilute to 25 ml with *water, Appendix 2.3.3.*
Chlorides –Dissolve 1.0 g in *water* with the addition of 2 ml of *nitric acid;* the solution complies with the *limit test for chlorides,* Appendix 2.3.2.

Sulphates –Dissolve 2 g in *water* with the addition of 2 ml of *hydrochloric acid;* the solution complies with the limit test for *sulphates,* Appendix 2.3.7.

Ammonium compounds –1 g warmed with 10 ml of *sodium hydroxide solution* does not evolve ammonia.

Assay –Weigh accurately about 1 g, dissolve in 20 ml of *water,* and titrate with 0.5 *N sulphuric acid* using *methyl orange solution* as indicator. Each ml of 0.5 *N sulphuric acid* is equivalent to 0.042 g of $NaHCO_3$.

Storage –Store in well-closed containers.

Sodium Bicarbonate Solution –A 5 per cnet w/v solution of *sodium bicarbonate* in *water.*

Sodium Bisulphite –Consists of sodium bisulphite ($NaHSO_3$) and sodium metabisulphite ($Na2S2O_3$) in varying proportions. It yields not less than 58.5 per cent and not more than 67.4 per cent of SO_2.

Description –White or yellowish-white crystals or granular powder; odour of sulphur dioxide. It is unstable in air.

Solubility –Freely soluble in *water,* slightly soluble in *alcohol.*

Assay –Weigh accurately about 0.2 g and transfer to a glass-stoppered flask, add 50 ml of 0.1 *N iodine* and insert the stopper of the flask. Allow to stand for five minutes, add 1 ml of *hydrochloric acid,* and titrate the excess of iodine with 0.1 *N sodium thiosulphate,* using *starch solution* as indicator added towards the end of the titration. Each ml of 0.1 *N iodine* is equivalent to 0.003203 g of SO_2.

Storage –Preserve Sodium Bisulphite in tightly-closed containers in a cool place.

Sodium Bisulphite Solution –Dissolve 10 g of *sodium bisulphite* in sufficient *water* to make 30 ml.

Sodium Bisulphite Solution must be freshly prepared.

Sodium Carbonate – $Na_2CO_3 . 10H_2O$ =286.2.

Analytical reagent grade.

Sodium Chloride – NaCl = 58.44

Analytical reagent grade.

Sodium Cobaltinitrite –$Na_3CO(NO_2)_6$ = 403.94

Description –An orange-yellow powder.

Solubility –Readily soluble in *water,* forming a clear orange-red solution.

Potassium – Dissolve 3 g in 10 ml of *water* , add the solution to a mixture of 5 ml of *water* and 2 ml of *dilute acetic acid,* and allow to stand for one hour; no precipitate is produced.

Sodium Cobaltinitrite Solution – A 30 per cent w/v solution of *sodium cobaltinitrite* in *water.*

Sodium Diethyldithiocarbamate –$(C_2H_5)_2$, N. CS.SNa, $3H_2O$ = 225.30.

Description –White or colourless crystals.

Solubility –Readily soluble in *water*, yielding a colourless solution.

Sensitivity –Add 10 ml of a 0.1 per cent w/v solution to 50 ml of *water* containing 0.002 mg of copper previously made alkaline with *dilute ammonia solution.* A yellowish-brown colour should be apparent in the solution when compared with a blank test containing no copper.

Sodium Diethyldithiocarbamate Solution – A 0.1 per cent w/v solution of *sodium diethyldithiocarbamate* in *water.*

Sodium Hydroxide –NaOH = 40.00

Description –White sticks, pellets, fused masses, or scales; dry, hard brittle, and showing a crystalline fracture, very deliquescent; strongly alkaline and corrosive.

Solubility –Freely soluble in *water* and in *alcohol.*

Aluminium, iron and matter insoluble in hydrochloric acid –Boil 5 g with 50 ml of dilute hydrochloric acid, cool, make alkaline with *dilute ammonia solution,* boil, filter, and wash with a 2.5 per cent w/v solution of *ammonium nitrate;* the insoluble residue after ignition to constant weight weighs not more than 5 mg.

Arsenic –Not more than 4 parts per million, Appendix 2.3.1.

Heavy metals –Not more than 30 parts per million, determined by Method A, Appendix 2.3.3. on a solution prepared by dissolving 0.67 g in 5 ml of water and 7 ml of 3 *N hydrochloric acid.* Heat to boiling, cool and dilute to 25 ml with water.

Potassium –Acidify 5 ml of a 5 per cent w/v solution with *acetic acid* and add 3 drops of *sodium cobaltnitrite solution;* no preciptitate is formed.

Chloride –0.5 g dissolved in *water* with the addition of 1.8 ml of *nitric acid,* complies with the limit test for *chlorides,* Appendix 2.3.2.

Sulphates –1 g dissolved in *water* with the addition of 3.5 ml of *hydrochloric acid* complies with the limit test for *sulphates,* Appendix 2.3.7.

Assay –Weigh accurately about 1.5 g and dissolve in about 40 ml of *carbon dioxide-free water.* Cool and titrate with *N sulphuric acid* using *phenolphthalein solution* as indicator. When the pink colour of the solution is discharged, record the volume of acid solution required, add *methyl orange solution* and continue the titration until a persistent pink colour is produced. Each ml of *N sulphuric acid* is equivalent to 0.040 g of total alkali calculated as NaOH and each ml of acid consumed in the titration with *methyl orange* is equivalent to 0.106 g of Na_2CO_3.

Storage –Store in tightly closed containers.

Sodium Hydroxide, xN – Solutions of any normality, xN may be prepared by dissolving 40 x g of *sodium hydroxide* in *water* and diluting to 1000 ml.

Sodium Hydroxide Solution – A 20.0 per cent w/v solution of *sodium hydroxide* in *water*.

Sodium Hydroxide Solution, Dilute –

A 5.0 per cent w/v solution of *sodium hydroxide* in water.

Sodium Nitrite –$NaNO_2$ = 69.00, Analytical reagent grade.

Sodium Nitroprusside –(Sodium penta cyano nitrosyl ferrate (iii) dihydrate; $Na_2[Fe(CN)_5 (NO)], 2H_2O$ = 298.0

Analytical reagent grade of commerce.

Sodium Peroxide – Na_2O_2 =77.98.

Analytical grade reagent.

Sodium Potassium Tartrate –Rochelle Salt COONa.CH(OH). CH(OH), COOK, $4H_2O$ = 282.17

Contains not less than 99.0 per cent and not more than the equivalent of 104.0 per cent of $C_4H_4O_6KNa, 4H_2O$.

Description –Colourless crystals or a white, crystalline powder; odourless; taste saline and cooling. It effloresces slightly in warm, dry air, the crystals are often coated with a white powder.

Solubility –Soluble in *water;* practically insoluble in alcohol.

Acidity or Alkalinity –Dissolve 1 g in 10 ml of recently boiled and cooled *water,* the solution requires for neutralisation not more than 0.1 ml of 0.1 *N sodium hydroxide* or of 0.1 *N hydrochloric acid,* using *phenolphthalein solution as indicator.*

Iron –0.5 g complies with the *limit test for iron,* Appendix 2.3.4.

Chloride –0.5 g complies with the *limit test for chlorides,* Appendix 2.3.2.

Sulphate –0.5 g complies with the *limit test for sulphate ,* Appendix 2.3.7.

Assay –Weigh accurately about 2 g and heat until carbonised, cool, and boil the residue with 50 ml of *water* and 50 ml of 0.5 *N sulphuric acid;* filter, and wash the filter with *water;* titrate the excess of acid in the filtrate and washings with 0.5 *N sodium hydroxide,* using *methyl orange solution* as indicator. Each ml of 0.5 *N sulphuric acid* is equivalent to 0.07056 g of $C_4H_4O_6KNa, 4H_2O$.

Sodium Sulphide –Na_2S + aq.

Analytical reagent grade. Deliquescent, crystalline masses turning yellow on storage.

Sodium Sulphide Solution –Dissolve with heating, 12 g of *sodium sulphide* in a mixture of 10 ml of *water* and 25 ml of *glycerol,* cool and dilute to 100 ml with the same mixture.

Sodium Sulphite, Anhydrous –Na_2SO_3 =126.06

Description –Small crystals or powder.

Solubility –Freely soluble in *water,* soluble in *glycerin;* almost insoluble in *alcohol.*

Sodium Thiosulphate – $Na_2S_2O_3$, $5H_2O$ =248.17.

Description –Large colourless crystals or coarse, crystalline powder; odourless; taste, saline, deliquescent in moist air and effloresces in dry air at temperature above 33º.
Solubility –Very soluble in *water;* insoluble in *alcohol.*

pH –Between 6.0 and 8.4, determined in a 10 per cent w/v solution.

Arsenic –Not more than 2 parts per million, Appendix 2.3.1.

Heavy metals –Not more than 20 parts per million, determined by Method A, Appendix 2.3.3. on a solution prepared in the following manner : Dissolve 1 g in 10 ml of *water,* slowly add 5 ml of *dilute hydrochloric acid* and evaporate the mixture to dryness on a water-bath. Gently boil the residue with 15 ml of *water* for two minutes, and filter. Heat the filtrate to boiling, and add *sufficient bromine solution* to the hot filtrate to produce a clear solution and add a slight excess of *bromine solution.* Boil the solution to expel the *bromine* completely, cool to room temperature, then add a drop of *phenolphthalein solution* and *sodium hydroxide solution* until a slight pink colour is produced. Add 2 ml of *dilute acetic acid* and dilute with *water* to 25 ml.

Calcium –Dissolve 1 g in 20 ml of *water,* and add a few ml of *ammonium oxalate solution;* no turbidity is produced.

Chloride –Dissolve 0.25 g in 15 ml of *2N nitric acid* and boil gently for three to four minutes, cool and filter; the filtrate complies with the *limit test for chlorides,* Appendix 2.3.2.

Sulphate and Sulphite –Dissolve 0.25 g in 10 ml of *water*, to 3 ml of this solution add 2 ml of *iodine solution,* and gradually add more *iodine solution*, dropwise until a very faint-persistant yellow colour is procduced; the resulting solution complies with the limit test for sulphates, Appendix 2.3.7.

Sulphide –Dissolve 1 g in 10 ml of *water* and 10.00 ml of a freshly prepared 5 per cent w/v solution of *sodium nitroprusside;* the solution does not become violet.

Assay –Weigh accurately about 0.8 g and dissolve in 30 ml of *water.* Titrate with 0.1 *N iodine,* using 3 ml of *starch solution* as indicator as the end-point is approached. Each ml of 0.1 iodine is equivalent to 0.02482 g of $Na_2S_2O_3$, $5H_2O$.

Storage –Store in tightly-closed containers.

Sodium Thiosulphate 0.1 N – $Na_2S_2O_3$, $5H_2O$. = 248.17, 24.82 g in 1000 ml.

Dissolve about 26 g of *sodium thiosulphate* and 0.2 g of *sodium carbonate* in *carbon dioxide-free water* and dilute to 1000 ml with the same solvent. Standardise the solution as follows :

Dissolve 0.300 g of *potassium bromate* in sufficient *water* to produce 250 ml. To 50 ml of this solution, add 2 g of *potassium iodide* and 3 ml of 2 *N hydrochloric acid* and titrate with the *sodium-thiosulphate solution* using *starch solution,* added towards the end of the titration, as indicator until the blue colour is discharged. Each 0.002784 g of *potassium bromate* is equivalent to 1 ml of 0.1*N sodium thiosulphate.* Note: –Re-standardise 0.1 *N sodium thiosulphate* frequently.

Stannous Chloride – $SnCl_2$, $2H_2O$ =225.63.

Contains not less than 97.0 per cent of $SnCl_2$, $2H_2O$.

Description –Colourless crystals.

Solubility –Soluble in *dilute hydrochloric acid.*

Arsenic- Dissolve 5.0 g in 10 ml of *hydrochloric acid,* heat to boiling and allow to stand for one hour; the solution shows no darkening when compared with a freshly prepared solution of 5.0 g in 10 ml of *hydrochloric acid.*

Sulphate –5.0 g with the addition of 2 ml of *dilute hydrochloric acid*, complies with the *limit test for sulphates,* Appendix 2.3.7.

Assay –Weigh accurately about 1.0 g and dissolve in 30 ml of *hydrochloric acid* in a stoppered flask. Add 20 ml of *water* and 5 ml of *chloroform* and titrate rapidly with 0.05 *M potassium iodate until* the *chloroform* layer is colourless. Each ml of 0.05 M potassium iodate is equivalent to 0.02256 g of $SnCl_2$, $2H_2O$.

Stannous Chloride Solution – May be prepared by either of the two methods given below :

1. Dissolve 330 g of *stannous chloride* in 100 ml of *hydrochloric acid* and add sufficient *water* to produce 1000 ml.

2. Dilute 60 ml of *hydrochloric acid* with 20 ml of *water*, add 20 g of tin and heat gently until gas ceases to be evolved; add sufficient *water* to produce 100 ml, allowing the undissolved tin to remain in the solution.

Starch Soluble – Starch which has been treated with *hydrochloric acid* until after being washed, it forms an almost clear liquid solution in hot water.

Description –Fine, white powder.

Solubility –Soluble in hot *water,* usually forming a slightly turbid *solution.*

Acidity or Alkalinity –Shake 2 g with 20 ml of *water* for three minutes and filter; the filtrate is not alkaline or more than fainthy acid to litmus paper.

Sensitivity –Mix 1 g with a little cold *water* and add 200 ml *boiling water.* Add 5 ml of this solution to 100 ml of *water* and add 0.05 ml of 0.1 *N iodine.* The deep blue colour is discharged by 0.05 ml of 0.1 *N sodium thiosulphate.*

Ash – Not more than 0.3 per cent, Appendix 2.2.3.

Starch Solution –Triturate 0.5 g of *soluble starch,* with 5 ml of *water,* and add this, with constant stirring, to sufficient water to produce about 100 ml. Boil for a few minutes, cool, and filter.

Solution of *starch must be recently prepared.*

Sudan Red G –Sudan III; Solvent Red 23; 1-(4-Phenyl-azophenylazo)-2-naphthol; $C_{22}H_{16}N_4O$ = 352.40.

Description –Reddish-brown powder.

Solubility –Insoluble in *water*; soluble in *chloroform,* in *glacial acetic acid;* moderately soluble in *alcohol,* in solvent *ether* and in *acetone*.

Sulphamic Acid –NH_2SO_3H =97.09.

Contains not less than 98.0 per cent of H_3NO_3S .

Description -White crystals or a white crystalline powder.
Solubility –Readily soluble in water.

Melting Range –203º to 205º, with decomposition.

Sulphuric Acid – H2SO4 = 98.08.

When no molarity is indicated use analytical reagent grade of commerce containing about 98 per cent w/w of *sulphuric acid.* An oily, corrosive liquid weighing about 1.84 g per ml and about 18 M in strength.

When solutions of molarity xM are required, they should be prepared by carefully adding 54 x ml of sulphuric acid to an equal volume of water and diluting with water to 1000 ml.

Solutions of sulphuric acid contain about 10 per cent w/v of H_2SO_4 per g mol.

Sulphuric Acid, Dilute –Contains approximately 10 per cent w/w of H_2SO_4.

Dilute 57 ml of sulphuric acid to 1000 ml with water.

Sulphuric Acid, Chlorine-free –Sulphuric acid which complies with the following additional test:

Chloride –Mix 2 ml with 50 ml of water and add 1 ml of solution of *silver nitrate,* no opalescence is produced.

Sulphuric Acid, Nitrogen-free-Sulphuric acid which contains not less than 98.0 per cent w/w of H_2SO_4 and complies with the following additional test :

Nitrate –Mix 45 ml with 5 ml of *water*, cool and add 8 mg of *diphenyl benezidine;* the solution is colourless or not more than very pale blue.

Tartaric Acid –$(CHOH.\ COOH)_2$ =150.1

Analytical reagent grade.

Thioglycollic Acid – Mercapto acetic acid, – HS. CH_2COOH =92.11.

Contains not less than 89.0 per cent w/w of $C_2H_4O_2S$, as determined by both parts of the Assay described below :

Description –Colourless or nearly colourless liquid; odour strong and upleasant.

Iron –Mix 0.1 ml with 50 ml of water and render alkaline with *strong ammonia solution;* no pink colour is produced.

Assay –

(i) Weigh accurately about 0.4 g and dissolve in 20 ml of *water* and titrate with 0.1 *N sodium hydroxide* using *cresol red solution* as indicator. Each ml of 0.1 *N sodium hydroxide is equivalent to* 0.009212 g of $C_2H_4O_2S$.

i. To the above neutralised solution and 2 g of *sodium bicarbonate* and titrate with 0.1 *N iodine* Each ml of 0.1 *N iodine is equivalent to 0.009212 g of* $C_2H_4O_2S$.

Thymol – 2-Isopropyl-5-methylphenol; $C_{10}H_{14}O$ = 150.2

General reagent grade.

Colourless crystals with an aromatic odour; freezing point not below 49º.

Thymol Blue –6, 6' –(3H-2, 1 Benzoxathil –3 –ylidene) dithymol SS =dioxide; $C_{27}H_{30}O_5S$ = 466.6

Gives a red colour in strongly acid solutions, a yellow colour in weakly acid and weakly alkaline solutions, and a blue colour in more strongly alkaline solutions (pH range, 1.2 to 2.8 and 2.0 to 9.6).

Thymol Blue Solution –Warm 0.1 g of *thymol blue* with 4.3 ml of 0.05 M sodium hydroxide and 5 ml of *ethanol (90 per cent);* after solution is effected add sufficient *ethanol (20 per cent)*to produce 250 ml.

Complies with the following test –

Sensitivity –A mixture of 0.1 ml and 100 ml of carbon dioxide-free water to which 0.2 ml of 0.02 *N sodium hydroxide* has been added is blue. Not more than 0.1 ml of 0.2 *N hydrochloric* acid is required to change the colour to yellow.

Titanous Chloride Solution –General reagent grade of commerce containing about 15 per cent w/v to TiCl3.

Weight per ml, about 1.2 g.

Dull purplish liquid with a strongly acid reaction.

Titanous Chloride 0.1 N – $TiCl_3$=154.26; 15.43 g in 1000 ml.

Add 103 ml of *titanous chloride solution* to 100 ml of *hydrochloric acid,* dilute to 1000 ml with recently boiled and cooled water, and mix, standardise, immediately before use, as follows :

Place an accurately measured volume of about 30 ml of standardised 0.1 *N ferric ammonium sulphate* in a flask and pass in a rapid stream of *carbon dioxide* until all the air has been removed. Add the *titanous chloride solution* from a burette and in an atmosphere of carbon dioxide until near the calculated end point then add 5 ml of *ammonium thiocyanate solution,* and continue the titration until the solution is colourless. Each ml of 0.1 *N ferric ammonium sulphate* is equivalent to 0.01543 g of $TiCl_3$.

Vanillin-Sulphuric Acid Reagent – 5 % Ethanolic sulphuric acid (Solution I)
1 % Ethanolic vanillin (Solution II)

The plate is sprayed vigorously with 10 ml Solution I, followed immediately by 5-10 ml of Solution II.

Water –See purified water.

Water, Ammonia-free –Water which has been boiled vigorously for a few minutes and protected from the atomosphere during cooling and storage.

Xylenol Orange – [3H-2,1-Benzoxathiol–3-ylidene bis – (6-hydroxy-5-methyl-m-phenylene) methylenenitrilo] tetra acetic acid SS-dioxide or its tetra sodium salt.

Gives a reddish-purple colour with mercury, lead, zinc and contain other metal ions in acid solution. When metal ions are absent, for example, in the presence of an excess of *disodium ethylenediamine tetraacetate*, this solution is yellow.

Xylenol Orange Solution –Shake 0.1 g of *xylenol orange* with 100 ml of *water* and filter, if necessary.

Zinc, Granulated –Zn=65.38.

Analytical reagent grade of commerce.

Zinc Powder –Zn =65.38.

Analytical reagent grade of commerce.

Zinc Sulphate –$ZnSO_4$, $7H_2O$ = 287.6.

Analytical reagent grade of commerce.

Appendix 5

GENERAL INFORMATION

(A) Definition and Method of Method of Preparing Kvātha or decoction

(Caraka Samhita, Sutrasthana Adhyaya 4; 8 1/2)

(Dravyaguna Vijnana, Paribhasakhanda)

Kvātha is the decoction obtained by boiling coarse powder of drug(s) in proportion of 4, 8 Or 16 times (for Mrdu dravya-4 times; for madhyama dravya -8 times and for khara dravya-16 times respectively) of water reduced to one-fourth and strained in cloth.

(B) Śōdhana of Drugs

Śōdhana is the process of grinding etc. with specified drugs for removal of impurities and potentiation of drugs.

The PARIBHASA of various ayurvedic technical terms used under Śōdhana are as follows

DOLAYANTRA

(Rasaratna Samucchaya, Adhyaya 9; 3-4)

Dolayantra is a contrivance consisting of a pot half filled with liquid with a horizontal rod put On the rim from which is suspended a quantity of the drug tied up in a thin cloth to be heated.

BHĀVANA

(Rasatarngini, Taranga 2; 49 & Bhaishaja Ratnavali Paribhasa Prakaran 50)

Bhāvana is the process by which powders of drugs are ground to a soft mass with liquid substances and are then allowed to dry under the sun rays during the day and are again put in the liquids during the night time. This process is repeated for 7 days unless otherwise mentioned.

NlMAJJANA

(Rasendracudamani, Adhyaya 4; 77)

NIMAJJANA is the process of Immersing the drug material in a specified liquid.

KĀÑJĪKA

(paribhasa paradipa)

Powder of Aśudhanya such as Kulmāsa Sasthika Rice, etc. along with a small quantity of white raddish (Mūlaka) cut into pieces 768 g. are placed in an earthen pot and 3 .072litres of water is added. The mo.ith of the pot is closed and kept for two, three weeks during which period the fluid becomes sour. This sour fluid is called Kāñjika, Dhanyāmla or Aranāla

CŪRṆAODAKA

(Rasatarangini, Taranga 11; 216-217)

The filtrate obtained by mixing 60 ml of water with 250 mg. of lime powder keeping for 9 hours and then filtering after decantation, is called Cūrnodaka or Sudhodaka.

PUTAPAKA
(Sarangadhara Samhita, Madhyama Khanda, Adhyaya I; 22-24 1/2)

Putapaka Svarasa is the juice of fresh drug obtained by the process of putapaka, The kalka (paste) of plant material is bundled in leaves of gambhari, vata, eranda, etc.

The bundle is covered with clay in layers of about 2 cm. thickness. When the clay is dried, the bundle is placed amidst fire till it becomes reddish. The bundle is then opened, juice from kalka is pressed out

METIIOD OF ŚODHANA :-

1. Citraka Śodhana
(Rasatarangini, Taranga 24; 575)

(1) Citraka mula (Rt.) I Part

(2) Curnodaka Q. S. for nimajjana.

Method-Small pieces of citrakamiila are soaked in lime water and thereafter washed, dried in the sun.

2. Guñjā Śodhana
(Rasarntta, Parisista 8; Page 147)

1. Guñjā(seed) 1 part

2. Kāñjīka Q.S for svedana.

Method- Svedana is done for three hours in Dōlāyantra. The testa is removed there after it is washed, dried and kept.

3. Hiṅgu Śodhana
(Rasatarangini, Taranga 24; 578)

3 Rāmaṭha(Hiṅgu) (Exd) 1 part

4 Ajya (Gogṛta) Q.S

Method- Hiṅgu is roasted with ghee in a pan till it becomes crisp.

4. Kampilla Śodhana
(Ayurveda Prakasa, Adhyaya 2; 346)

1.Kampilla (phala raja) 1 Part
2.Matulunga rasa (Fr.) Q. S. for bhavana
3. Ardraka rasa (Rz.) Q. S. for bhavana

Method-Kampillaka available in the market is heavily adulterated (mixed with brick powder or more commonly with tile powder. The material therefore is to be briskly shaken with water so that the tile powder settles down & Kampillaka Raja float on the top of water. It is to be removed out allowing the settled matter undisturbed. This may be repeated 3 times and the top layer (very light) is allowed to dry and subjected to the Śodhana.

Bhāvana is given three times with ingredient no. 2 & 3 separately.

5. Karavīra Śodhana
(Sarangadhara samhita, Madhyama khanda

1. Karavīra (lf.)	1 part
2. Godugdha	Q.S. for svedana

Method- Svedana is done in Dōlāyantra for three hours

6. Snuhī Śodhana
(rasatarangini, Taranga 24; 517-518)

1. Sudha (Snuhī) (St.)	48g
2. Ciñca dala drava	24

Method- Ciñca Patra svarasa 9obtained from Puṭapāka) is mixed with powder of Snuhī and dried in the sun

7. Vijayā Śodhana
(Rasamṛta Pariśiṣṭa 8; page 147)

ii.	Vijaya (lf)	Part 1
iii.	Jala	Q.S of Prakṣālana

Method- the leaves of Vijayā are put in muslin bag and washed in water till free from turbidity and then dried.

APPENDIX –6

6.1 WEIGHT AND MEASURES

METRIC SYSTEM

Measure of Mass (Weights)

1 Kilogram (Kg) – is the mass of the International Prototype Kilogram.
1 Gramme (g) – the 1000th part of 1 Kilogram.
1Milligram (mg) – the 1000th part of 1 gramme.
1 Microgram (µg) – the 1000th part of 1 milligram.

Measures of capacity (Volumes)

1 Litre (1) is the volume occupied at its temperature of maximum density by a quantity of water having a mass of 1 Kilogram.

1 Millilitre (ml) the 1000th part of 1 litre.

The accepted relation between the litre and the cubic centimetre is 1 litre –1000.027 cubic centimeters.

Relation of capacity of Weight (Metric)

One litre of water at 20º weighs 997.18 grammes when weighed in air of density 0.0012 gramme per millilitre against brass weights of density 84 grammes per millilitre.

Measures of Length

1 Metre (m) is the length of the International Prototype Metre at 0.
1 Centimetre (cm) – the 100th part of 1 metre.
1 Millimetre (mm) – the 1000th part of 1 metre.
1 Micron (µ) – the 1000th part of 1 millimetre
1 Milliimicron (mµ) – the 1000th part of micron.

5.2 APPROXIMATE EQUIVALENTS OF DOSES IN INDIAN SYSTEM AND METRIC SYSTEM :

1	Ratti or Gunja		=125 mg
8	Rattis or Gunjas	=1 Masa	=1 g
12	Masa	=1 Karsa (Tola)	=12 g
2	Karsas (Tolas)	=1 Sukti	=24 g
2	Suktis (4 Karsas or Tolas)	=1 Pal	=48 g
2	Palas	=1 Prasrti	=96 g
2	Prasrtis	=1 Kudava	=192 g
2	Kudavas	=1 Manika	=384 g
2	Manikas	=1 Prastha	=768 g
4	Prasthas	=1 Adhaka	=3 Kg 73 g
4	Adhakas	=1Drona	= 12 Kg 288 g
2	Dronas	=1Surpa	= 24 Kg 576 g
2	Surpas	=1 Droni (Vahi)	= 49 Kg 152 g
4	Dronis	=1 Khari	=196 Kg 608 g
100	Palas	=1 Tul¡	= 4 Kg 800 g
20	Tul¡s	=1 Bhara	= 96 Kg